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# **EPIDEMIOLOGY AND TREATMENT OPTIONS OF PRIMARY BILIARY CIRRHOSIS**

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*To my family*

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## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

- I Rautiainen H, Salomaa V, Niemelä S, Karvonen A-L, Nurmi H, Isoniemi H, Färkkilä M. Prevalence and incidence of primary biliary cirrhosis are increasing in Finland. *Scand J Gastroenterol* 2007;42:1347-1353.
- II Rautiainen H, Kärkkäinen P, Karvonen A-L, Nurmi H, Pikkarainen P, Nuutinen H, Färkkilä M. Budesonide combined with UDCA to improve liver histology in primary biliary cirrhosis: A three-year randomized trial. *Hepatology* 2005;41:747-52.
- III Rautiainen H, Färkkilä M, Neuvonen M, Sane T, Karvonen A-L, Nurmi H, Kärkkäinen P, Neuvonen PJ, Backman JT. Pharmacokinetics and bone effects of budesonide in primary. biliary cirrhosis. *Alimentary Pharmacology and Therapeutics* 2006;24:1545-1552.
- IV Färkkilä M, Rautiainen H, Kärkkäinen P, Karvonen A-L, Nurmi H, Niemelä O. Serological markers for monitoring disease progression in non-cirrhotic PBC on UDCA therapy. *Liver International*. In press.

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## ABBREVIATIONS

AIC	autoimmune cholangitis
AIH	autoimmune hepatitis
ALB	albumin
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMA	antimitochondrial antibodies
ANA	anti nuclear antibodies
AST	aspartate aminotransferase
APRI	AST/platelet ratio index
AUC	area under the concentration
AUROC	area under receiver operating characteristic (ROC)
BA	bile acid
BIL	bilirubin
BMD	bone mass density
BMI	body mass index
BX	biopsy
Campe	campesterol
CI	confidence interval
C <sub>max</sub>	peak concentration
CNS	central nervous system
CRT	controlled randomized trial
CV	coefficient of variation
ERS	erythrocyte sedimentation rate
FN	femoral neck
FB-GLUC	fasting blood glucose
GT	λ- glutamyl-traspeptidase
HR	hazard ratio
HBV	hepatitis B
HCV	hepatitis C
HA	hyaluronic acid
HBsAg	hepatitis-B-surface-antigen
HCV-ab	hepatitis-C-antibody
IgG	immunoglobulin G
IgM	immunoglobulin M
LKM	liver-kidney-microsomal antibodies
LPN	lymfocytic piecemeal necrosis (interface inflammation)



LS	lumbar spine
NTX	urinary-N-telopeptide-collagen
2-OACD	2-oxoglutaric dehydrogenase complex
PDC-E2	Pyruvate dehydrogenase dihydrolipoamine acetyltransferase
PBC	primary biliary cirrhosis
PSC	primary sclerosing cholangitis
PTH	parathyroid hormone
ROC	receiver operating characteristic
PIIINP	amino-terminal propeptide of type III procollagen
SFS	super fund toxic waste sites
Sito	sitosterol
SMA	smooth muscle antibodies
TIMP1	tissue inhibitor of matrix metalloproteinase 1
UDCA	ursodeoxycholic acid

# ABSTRACT

**Background:** The prevalence and incidence of primary biliary cirrhosis (PBC) is increasing in the Western world. The highest prevalence, 402 per million, is reported in the USA and the highest incidence in Scotland, 49 per million per year. Our aim was to assess the epidemiology of PBC in different areas of Finland and to find out whether the possible increase is related to better survival or increased incidence, or both.

The treatment of PBC is based on Ursodeoxycholic acid (UDCA). All of the patients, however, do not receive biochemical and histological remission with this therapy. Our treatment option was to combine budesonide, a potent corticosteroid with a high first pass metabolism in the liver, to UDCA and determine whether the suppression of inflammation and fibrosis is achievable and for safety reasons investigate the bone effects and pharmacokinetics of budesonide in PBC patients.

The liver histology has been the only reliable way to assess and follow the stage and inflammation grade of PBC. Our aim was to find out if any laboratory test, or combination of tests, would serve as a surrogate marker for liver fibrosis or inflammation.

**Patients and methods:** Patients for the epidemiological study were searched from the hospital discharge records from year 1988 to 1999. The diagnosis was confirmed from hospital case records, the place of residence from the Population Information System, and the deaths from the National Causes of Death Register. The population examined represents over 56% of the Finnish population.

In the treatment study, 77 PBC patients at stage I to III were randomized from 3 university hospitals to receive budesonide 6 mg/day combined to UDCA 15 mg /kg/day or UDCA alone for three years. The liver histology, bone mass density (BMD) measurement, Doppler ultrasound of the liver, and upper endoscopy with bile acid sample from duodenum were performed prior the treatment and at the end of the study to confirm the stage of the PBC and to find out the efficacy and side-effects of the treatment. Biochemistry was obtained with every visit at four months intervals. The pharmacokinetic study consisted of 22 patients whose 24 h bloodsamples were collected after ingestion of budesonide. The plasma budesonide concentrations were quantified by liquid chromatography-tandem mass spectrometry.

Sixty-nine paired biopsies were analyzed along with the patients routine biochemical markers, hyaluronic acid (HA), bile acids, cholesterol

precursors, plant sterols, and amino-terminal propeptide of type III procollagen (PIIINP) together with surrogate indexes: Forn's index, AST/platelet ratio index (APRI), Fibrosis index, PBC score.

**Results:** The prevalence of PBC rose from 103 (CI 97 to 110) to 180 (172–189) per million from 1988 to 1999, an annual increase of 5.1%. The incidence rose from 12 (10 to 14) to 17 (15 to 20) accordingly, an annual increase of 3.5%. The age at diagnosis remained stable (58–56 years), but the age at death increased markedly from 65 to 76. During the study period 53% of the deaths were liver related and 7% of them were due to hepatocellular carcinoma. The risk of death, especially liver related deaths, diminished over time. Hazard ratios were 0.6 (0.4–0.9) and 0.4 (0.2–0.8) per 10-year increment in time at PBC diagnosis.

The combination therapy with UDCA and budesonide was effective: stage improved 22%, fibrosis 25%, and inflammation 32%. In the UDCA group the changes were: 20% deterioration in stage and 70% in fibrosis, but a 10% improvement in inflammation. The comparison between the treatment groups were statistically significant for stage ( $p = 0.009$ ) and fibrosis ( $p = 0.0009$ ), but not for the inflammation.

BMD in femoral neck decreased by 3.6% in the combination group ( $p = 0.0002$ ) and by 1.9% in the UDCA group ( $p = 0.029$ ). The reductions in lumbar spine were 2.8% ( $p = 0.003$ ) and 0.7% ( $p = 0.25$ ), accordingly. The changes in BMD between the groups were not statistically different.

The mean s-cortisol values became significantly lower after two years in the combination group compared to the UDCA group, whereas the blood glucose levels did not differ.

The  $C_{\max}$ , AUC (0–24h), and  $t_{1/2}$  of budesonide did not differ significantly between the stages 0–I, II, and III, although the AUC (0–24h) seemed to be lower at stage 0. No statistically significant correlations between the  $C_{\max}$ , AUC (0–24h) or  $t_{1/2}$  of budesonide, and change of histological stage and grade, bilirubin level or change of BMD were found.

In precirrhotic PBC HA, PIIINP, bile acids, and AST were significantly different within stages I–III and could differentiate the mild fibrosis (F0F1) from the moderate (F2F3). The combination of these individual markers (PBC-score) further improved the accuracy ( $p < 0.0001$  in baseline stage,  $p < 0.001$  in baseline fibrosis). The accuracy of APRI at baseline was somewhat lower in stages ( $p < 0.01$ ) and in fibrosis ( $p < 0.05$ ). The area under the ROC of the PBC score, using a cut of value 66, had a sensitivity of 81.4% and a specificity of 65.2% to classify the stage of PBC. Previously reported scores did not reach significance in AUROC.

**Conclusions:** The epidemiology of PBC in Finland follows the increasing trend of other western countries. The prevalence and incidence resemble data from other Nordic countries and do not rise as high as prevalence

from USA and UK. The increasing prevalence in Finland is due to both increasing incidence and improved survival.

The combination of budesonide and UDCA improves liver histology compared to UDCA in non-cirrhotic stages of PBC. The treatment may lead to some systemic corticosteroid side-effects. The adrenal suppression and effects on bone has to be considered during the treatment. The pharmacokinetics of budesonide in different non cirrhotic stages of PBC is equal, although they may differ from the healthy liver. The budesonide and UDCA combination treatment is an option to those patients who do not receive full response from UDCA alone and are still at the non-cirrhotic stage of PBC.

Hyaluronic acid, PIIINP, AST, and bile acids may serve as tools to monitor the treatment response in the early stages of PBC. Combining these biomarkers in to a simple index potentiates their diagnostic value and gives possibilities of reducing repeated liver biopsies in patients' follow up.

# 1 INTRODUCTION

Primary biliary cirrhosis (PBC) is an autoimmune liver disease of unknown etiology. An inflammation in the small intrahepatic bile ducts leads to destruction of bile ducts, accumulation of toxic compounds in the liver, and eventually to cirrhosis. The incidence and prevalence of PBC are increasing in the western world. The reasons for increasing prevalence might be the increasing exposure of different chemicals in our everyday life, which may lead to the loss of tolerance in susceptible individuals. So far, there has been no epidemiological data of any cholestatic disease in Finland.

The treatment of PBC is based on administration of ursodeoxycholic acid (UDCA), which decreases inflammation in early PBC. Despite UDCA treatment, in 20 years time, over 50% of patients will end up with cirrhosis if other causes do not limit survival. Multiple other drugs have been tested for PBC, but none of the immunomodulators, for example, have fulfilled safety requirements nor shown enough effectiveness to become an additional drug of choice.

This thesis aims to find out the prevalence and incidence of PBC in Finland and the factors associated for the possible changes in the epidemiology of PBC.

Another aim was to find out whether the budesonide, a corticosteroid with high first pass metabolism, could stop the inflammation in small bile ducts and become an additional therapy to PBC. The safety aspects, including cortisol and bone metabolism, together with pharmacokinetic parameters necessitate careful investigation. The follow up of PBC has been based on the liver histology and our aim was to find out surrogate markers to make the assessment of the disease easier and safer.

## 2 REVIEW OF THE LITERATURE

Primary biliary cirrhosis (PBC) is an autoimmune liver disease with 90% female predominance. It is characterized by destruction of the bile duct epithelial cells in the small intrahepatic bile ducts. The loss of bile ducts leads to accumulation of toxic substances within the liver, which causes hepatic damage, fibrosis, cirrhosis, and liver failure. Nowadays, 60% of patients are asymptomatic at presentation (1). In symptomatic patients, the most common complaints are fatigue 21% and itching 19% (2).

### 2.1 Diagnosis and differential diagnosis

The diagnosis of PBC is based on the cholestatic serum enzyme pattern (elevated alkaline phosphatase), a compatible liver histology, and a high titer of antimitochondrial autoantibodies. The diagnosis is regarded definite if all three features exist and probable if two of them are present. The elevated IgM supports the diagnosis of PBC, but it is not always there.

#### 2.1.1 Histology

The histology of PBC consists of damaged or destroyed small bile ducts (less than 70–80  $\mu\text{m}$ ) accompanied by surrounding inflammation of the lymphocytes, plasma cells, eosinophils, and histiocytes. Granulomas may exist especially in the early phases of PBC.

The histological appearances are divided to four stages: Stage I, inflammation of portal triads; Stage II, reduced number of normal bile ducts and inflammation extending from the portal triads to the surrounding parenchyma; Stage III, fibrous septa linking adjacent portal triads; and Stage IV, cirrhosis with regenerative nodules (161). Changes in the liver are focal and different stages may overlap.

The inflammation activity can be graded (0–3) by the METAVIR point score (162) which is based on the combination of lobular and interface inflammation. Lobular inflammation is graded: 0 = less than one focus, 1 = one focus per lobule and 2 = multiple foci per lobule or bridging necrosis. Interface inflammation: 1 = focal in some portal areas, 2 = focal in most portal areas or diffuse in some, 3 = diffuse in all portal areas.

#### 2.1.2 Anti mitochondrial antibodies

The destruction of the bile duct epithelial cells in the small intrahepatic bile ducts is a T-cell mediated process. The targets of T-lymfocytes are

the dihydrolipoamine acetyltransferase components of the 2-oxo-acid-dehydrogenases in the mitochondrial inner leaf. Pyruvate dehydrogenase is the best known of these antigens and its dihydrolipoamine acetyltransferase component is PDC-E2. Ninety percent of patients have positive antimitochondrial autoantibodies (AMA) against PDC-E2 (3). With more sensitive recombinant methods for detecting AMA, the proportion of AMA negative patients decreases to 5% (4). When detecting anti PDC-E2, anti branched –chain  $\alpha$ -ketoacid dehydrogenase complex (BOADC-E2), and the 2-oxoglutaric-dehydrogenase complex (2-OADC) of those initially AMA negative patients 20% are positive for one or more of these antigens (5). The titer of AMA does not predict the stage of PBC nor does it reflect the effect on the treatment (6). The AMA negative PBC or autoimmune cholangitis (AIC) have the same disease features as PBC and they are usually antinuclear antibody (ANA) positive (5). Positive ANA are found in approximately 50% of PBC patients (7).

### **2.1.3 Differential diagnosis**

Differential diagnosis within autoimmune liver diseases is not always possible because of their overlapping tendency. The PBC-autoimmune hepatitis (AIH) combination appears in 8% (8) to 9% (9) of the cases and in the latest report upto 13% (10). PBC and primary sclerosing cholangitis (PSC) overlapping is a much rarer occasion than overlapping with AIH, but overlapping occurs (11) and also coexistent PBC and PSC are reported (12).

Other autoimmune conditions are common in PBC. In a study from UK, 53% of the patients had other autoimmune condition: Sjögren's syndrome 25%, autoimmune thyroid disease 23%, rheumatoid arthritis 17%, scleroderma 8%, Raynaud's phenomenon 24%, systemic lupus erythematosus (SLE) 1%, autoimmune thrombocytopenic purpura 1%, and pernicious anemia 4% (13).

## **2.2 Pathogenesis**

### **2.2.1 Genetics**

Familial clustering and high concordance in monozygotic twins, 63%, suggests PBC has a genetic component (14). The number of family members having PBC varies from 2.4% to 7.1% in studies published after 1990 (15). In a study from the UK the relative risk for a sibling of an affected individual to be diagnosed with PBC was 10.5 (16). A 1/10 female predominance exists, the reason for this is not completely understood. Women with PBC, however, have an increased prevalence for monosomy X in peripheral white blood cells (17). The loss of the X chromosome increases with age, which may explain the late onset of disease (17). Genes involved

in immunological tolerance are located in the X chromosome and loss of these genes may predispose to the breakdown of self-tolerance and to the development of autoimmune diseases (18). Microchimerism does not have role in PBC (18).

Despite efforts, consistent associations between PBC and HLA alleles have been difficult to find (15). From the non-MCH genes single nucleotide polymorphisms of 1,25-dihydroxyvitamin D receptor (VDR) and cytotoxic T lymphocyte antigen-4 (CTLA-4) have been implicated in PBC susceptibility. The association between HLA class I molecules and PBC seems weak, but some association for HLA-B alleles are found (19).

HLA class II association, HLA-DRB1\*0801, for PBC is found in caucasians in USA and in Europe (20–24). Some European studies also suggested an association with DR3 and DPB1\*0301 (25–27). The protective effect of the DRB1\*11 allele against PBC was found in Italian population (28,24) and DRB1\*13 in Italian and UK patients (24). HLA class II involvement in PBC is complex and the HLA-II background might follow a geographical pattern (15).

The genes of the MCH class III region include tumor necrosis factor alfa. Data from association studies for PBC are conflicting (29). A polymorphism of the gene promoter region more frequently produces variant the TNF1 and seldom TNF2, the latter is associated with increased transcription. It has been suggested that TNF2 allele would be protective against onset of PBC (30), but many studies have not been able to confirm this, nor find any association with TNF1/TNF2 and severity of PBC (15).

Single nucleotide polymorphisms of the 1,25-dihydroxyvitamin D receptor (VDR) gene is associated with PBC multiple studies (15). The VDR gene determines immunomodulatory activity of vitamin D. Its association with accelerated bone loss in cholestasis is suggested (31), but not confirmed (32). Certain alleles of VDR might explain the low prevalence of PBC in black women and theoretically exposure to sunlight may have a role in PBC (15).

Cytotoxic T lymphocyte antigen-4 (CTLA-4) is expressed by T cells when regulating peripheral T cell responses. Polymorphism is associated with PBC in England and in China (33–34).

### **2.2.2 Environmental factors**

The suspected mechanism for initiation of autoimmunity in PBC is molecular mimicry between microbial agents and self antigens (35). Environmental chemicals are metabolized primarily in the liver. The possible mechanisms of xenobiotics include direct toxic effect leading to abnormal cell death by apoptosis or necrosis, which may generate immunogenic auto-epitopes. Other mechanisms suggest that neoantigen-specific primed T and B cells crossreact with less immunogenic native autoantigen and/or chemical modification of the native cellular protein lead to presentation of



cryptic peptides (36). Environmental factors like retroviruses, chemicals (37), and bacteria for example *Escherichia coli* (38) have been suggested as causative agents. The latest and the strongest candidate for immunological trigger is *Novosphingobium aromaticivorans*, which has a close homology with human PDC-E2. *Novosphingobium aromaticivorans* is a gram negative aerobic bacterium found worldwide in soil, water, and coastal plain sediments (39). It can metabolize chemical compounds that are similar to xenobiotics, which are reactive against sera from PBC patients (37). Thus, *N. aromaticivorans* can break down self-tolerance by molecular mimicry due to subclinical infection and by metabolism of xenobiotics. In the initial study from Italy, 100% of the sera of AMA positive (anti PDC-E2), 33% of anti-branched-chain  $\alpha$ -ketoacid dehydrogenase complex (BOADC E2) positive, and even 12% of AMA negative PBC patients reacted against proteins from *N. aromaticivorans*. None of the control sera reacted against *N. aromaticivorans*. Twenty-five percent of the PBC patients and 25% of the controls had *N. aromaticivorans* in their feces (40). The finding was confirmed in the Icelandic population (41).

In a questionnaire based study in the USA smoking, tonsillectomy, and vaginal or urinary tract infections in females were associated with PBC compared with controls (42). This finding was confirmed with a larger interview based study from USA. The history of urinary tract infections, past smoking, and use of hormone replacement therapy were risk factors for PBC and slight association for use of nail polish was found (43). Smoking, urinary tract infections, itching at pregnancy, and dyed hair were risk factors for PBC in a questionnaire study from the UK (44). Cigarette smoke contains benzene, which is one of the volatile compounds found in super fund toxic waste sites (SFS). Living near these places increases the probability of ending up with a liver transplantation because of PBC. Chemical compounds may trigger autoimmunity and increase the risk for PBC or enhance the progression of PBC (45).

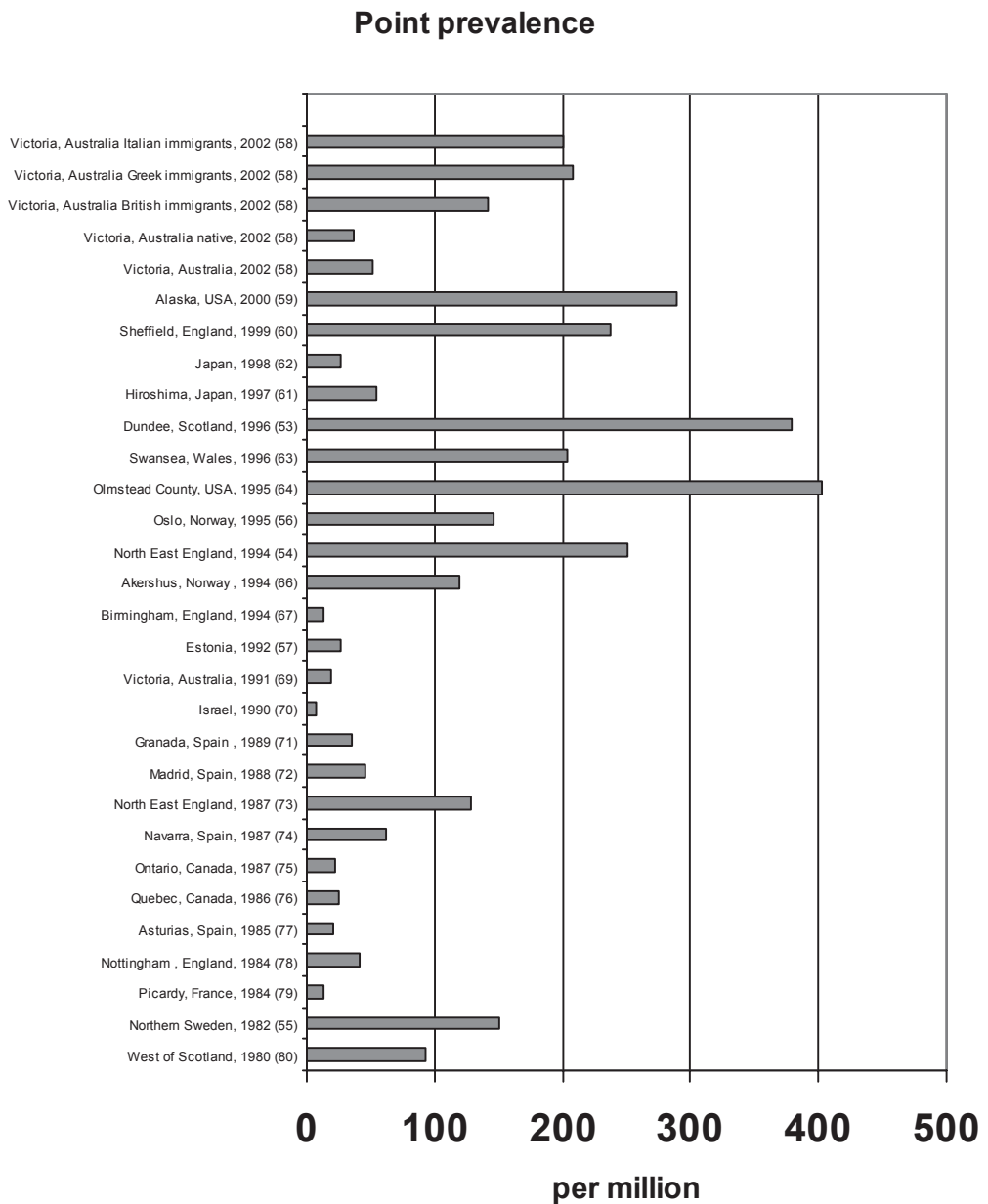
Common chemical reagents, aromatic and halogenated hydrocarbons may lead to autoantigen modifications and enhance autoantibody reactivity as well. For example 2-octunoid acid, widely used in the environment including perfumes, lipsticks, and many food flavorings, was found to modify PDC-E2 (46) and may perhaps be one of the explaining factors for female predominance of PBC.

## 2.3 Epidemiology

PBC mostly affects women in industrialized countries and the first epidemiological data on PBC were published 1974 (47), since that over forty epidemiological studies, mainly from industrialized countries, have been published. Many of the studies, especially those published before 1986,

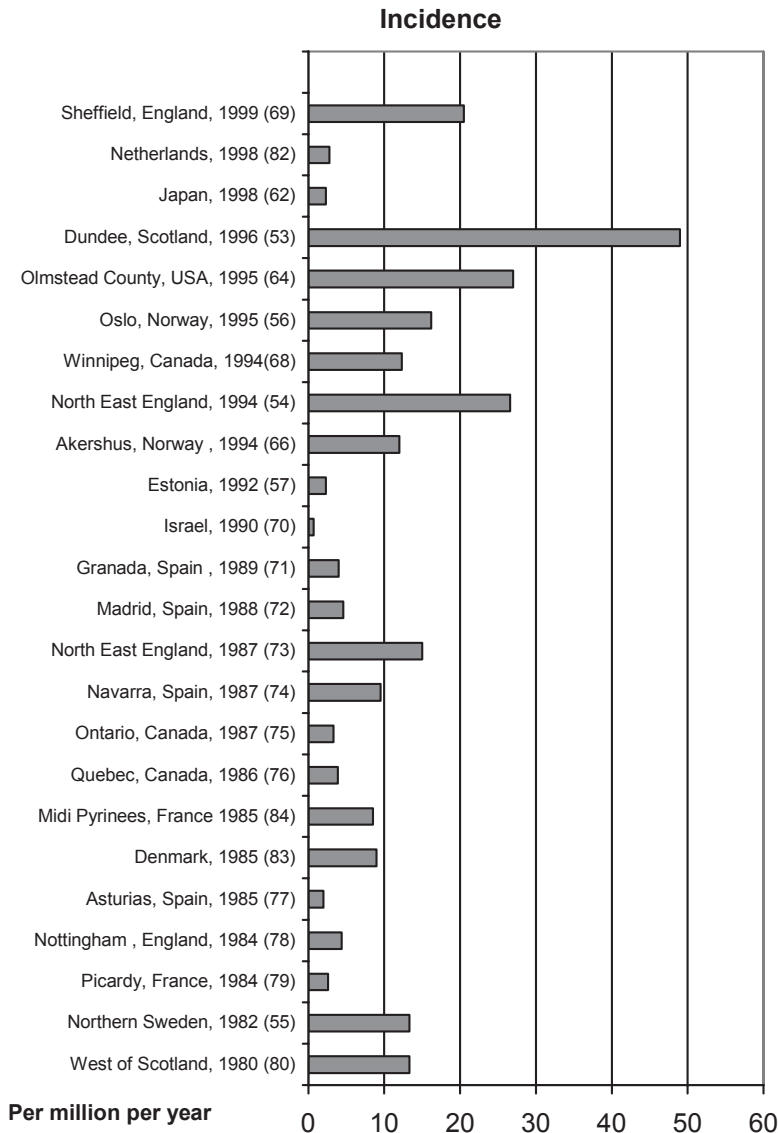
are of low quality (48). The sample sizes, case finding methods, diagnostic criteria, and reference populations vary greatly, which make it difficult to compare prevalence and incidence data in different studies. Prince M (48) has collected data in to a review article from epidemiological studies until 2001. In data before 1986 the highest incidence was found in Sweden, 13.7 per million per year (49,50), and the lowest in England and Wales, 0.6 per million per year (47). The highest prevalence was found in Sweden, 128 per million (50), and the lowest in Spain, 11.1 per million (51).

The epidemiological studies published from 1986 to 2002 are summarized in the figures 1 and 2. The highest prevalence among the whole population is reported from Olmsted county, Minnesota, in the USA, a prevalence of 402 per million (52). In Europe, the highest prevalences come from the UK, 379 per million in Scotland (53) and 251 in Newcastle, England (54). In northern Europe, epidemiological data on prevalence of PBC exist from Sweden, Norway, and Estonia. The prevalence was 151 per million in Sweden (55), 146 in Norway (56), and 27 in Estonia (57).



**Figure 1.** Point prevalence in order of year recorded. Values are obtained from a review article of Prince M & James OF 2003(48) until year the 2000 and after that from original articles.

Incidence rates of PBC are highest in the UK and USA, 49 per million per year in Scotland (53), 27 in Newcastle (54), and 27 in the USA (52). In Northern countries the incidence rates are 16 per million per year in Norway (56), 13 in Sweden (55), 9 in Denmark (81), and 2 in Estonia (57).



**Figure 2.** The mean incidence of different study periods in individual studies, the studies appear in order of the last year of survey. Values are obtained from a review article by Prince M & James OF 2003 (48) and after that from original articles.

## 2.4 The natural history of PBC

### 2.4.1 The natural history

Before 1970's the majority of the patients, 80%, were diagnosed when they were symptomatic (86) and most of them already had cirrhosis. In later years asymptomatic PBC patients were also diagnosed and their prognosis was better than that of those who had symptoms. It was also suggested that prognosis could be similar in age and sex matched control population (87).

Nowadays, 60% of patients are asymptomatic at presentation (86). The untreated asymptomatic PBC patients tend to develop symptoms within four years and their survival is lower than survival of the healthy population (88). In the cohort of 279 untreated patients the median survival of asymptomatic patients was 16 years and 7.5 years for symptomatic patients (89). Of asymptomatic patients, 33% remained symptom free in a median follow-up of 12 years but, after symptoms developed, the survival rates were similar to symptomatic patients. In a study of 91 patients followed up to 17 years confirmed the shorter survival for asymptomatic patients compared with the healthy control population (90), but those patients who remained asymptomatic had a similar survival compared with a matched control population. In that study they could not identify any prognostic features to find patients who would develop symptoms. A survival study of 770 patients from Northeast England shows no benefit for asymptomatic patients, 61% of patients were asymptomatic at diagnosis and their median survival was 9.6 years and survival for symptomatic patients was 8 years (91). The study is compromised of a high number of deaths in asymptomatic patients implicating non-hepatic causes of death and putting the age at diagnosis in a determining position.

The histological progression is divided into four stages (I to IV) and the stages predict the survival. The data from 916 biopsy specimens from 222 patients shows a histological progression during a median of 3 years of follow-up. From the patients presenting with stage I, 31% had developed cirrhosis in four years and of patients initially at stage II, 50% reached cirrhosis. The histology was stable in only 20% of patients and 2 % showed regression. The overall progression was one stage in every 1.5 years (92). A subgroup of patients who may develop profound cholestasis without portal hypertension or cirrhosis while protein synthesis is intact exists. These patients are ductopenic, but their liver is not fibrotic (93).

Bleeding from esophageal varices is a strong prognostic marker. The histological stage and elevated serum bilirubin are positive predictors for development of varices. Once varices have developed the 1 and 3 years survival rates are 83% and 59%, if bleeding has occurred the survival rates decrease to 65% and 46% (21). On the other hand, the varices may also be the first marker of disease progression; the development of varices in asymptomatic patients led to symptoms in 3 years (94).

Hepatobiliary malignancies, mainly hepatocellular carcinoma (HCC), affect patients with PBC. The relative risk was 46 in women and 55 in men in a retrospective analysis from the Mayo clinic (95). The probability of HCC in patients at stages III and IV PBC was similar to hepatitis C cirrhosis, but lower at earlier stages in a Spanish study (96). In a Japanese study this finding was confirmed and they reported a 7.7% cumulative appearance of HCC in 10 years at early stages and 12.3% advanced stages (97). Risk factors for HCC were age at the time of diagnosis, male gender, and a history of blood transfusions. In addition to these risk factors signs of portal hypertension and cirrhosis were associated with HCC in a study from Mayo clinic (98).

**2.4.2 Prognostic models and serological markers for monitoring PBC**

Three prognostic models of PBC are presented in Table 1. The Mayo model is based on 418 untreated patients and its advantage compared with two other models is its independence of liver histology. The major determinant of the Mayo model is bilirubin and the four other independent prognostic variables are patient’s age, serum albumin, prothrombin time, and the severity of fluid retention (99). It is useful in predicting cirrhosis and the need for a liver transplantation, but in non-cirrhotic PBC patients with normal bilirubin values the Mayo model does not detect progression of the stage.

**Table 1. Natural prognostic models in PBC and markers in liver fibrosis. (87,99–104)**

	Age	Fibrosis/ Cirrhosis	Bil	Ast	Cholesterol	Alt	HA	GT	Platelet	Prothrombtime	Alb	Edema	Cholestasis	Hepatomegaly
Yale, 1983 (87)	x	x	x											x
European, 1985 (100)	x	x	x								x		x	
Mayo, 1989 (99)	x		x							x	x	x		
Forns’ score, 2002 (101)	x				x			x	x					
APRI, 2003 (102)				x					x					
Fibrosis index, 2004 (103)			x				x							
Ast/alt, 2006 (104)				x		x								

In hepatitis C several non-invasive markers for evaluating fibrosis have successfully been introduced, for example Forn's score (101) and the AST/platelet ratio index (APRI) (102). In PBC the ALT/AST-ratio (104) and the sum of bilirubin and hyaluronic acid (Fibrosis index) (103) have recently showed some diagnostic value for detection of fibrosis, but not for lymphocytic piecemeal necrosis, previously shown to be associated with the risk of cirrhosis (105).

From serum non-cholesterol sterols, S-cholestanol, a 5 $\alpha$ -saturated derivate of cholesterol, has previously been shown to correlate closely with S-bilirubin and with the histological stage of primary biliary cirrhosis (106). Both intestinal absorption and biliary excretion of sito- and campesterols are regulated by ABCG5/8-transporters (107, 108) and the excretion of plants sterols are impaired in cholestasis, especially that of sitosterol (109). Therefore, non-cholesterol sterols and serum campe/sitosterol ratio have been suggested to serve as sensitive markers for developing cholestasis and the progression of PBC.

## 2.5 Treatment

### 2.5.1 Medical treatment

#### 2.5.1.1 *Ursodeoxycholic acid (UDCA)*

UDCA is hydrophilic tertiary bile acid, a 7 beta-epimer of chenodeoxycholic acid. UDCA constitutes only 1 to 3 per cent of biliary bile acids in man. In cholestasis, bile accumulates in the liver and the hydrophilic UDCA is less hepatotoxic than the more hydrophobic primary bile acids chenodeoxycholic acid and cholic acid.

Oral administration of UDCA 10–15 mg/kg/day leads to UDCA becoming a predominant circulating bile acid (40–60%), while the amount of cholic acid, chenodeoxycholic acid, and 3-beta -hydroxy-5 chenolenoic acid decrease (110).

Mechanisms of action of UDCA are multiple aiming at one or more pathogenetic processes in cholestasis. UDCA protects cholangiocytes against toxic effects of hydrophobic primary bile acids. The membrane protective effect is mainly at the bile duct level, where the bile acid concentrations are high. This effect is apparent in liver histology, where the portal inflammatory reaction is less severe in UDCA treated PBC patients than in placebo treated patients (111). The enhancement of impaired biliary secretion caused by UDCA, results from the stimulating expression of transporter proteins in the liver and insertion of transporter molecules into the canalicular membrane at transcriptional and posttranscriptional level (112,113). These mechanisms also increase the elimination of toxic biliary compounds. The antiapoptotic mechanisms of UDCA are

associated with a reduction of the mitochondrial membrane permeability transition and the mitochondrial cytochrome c release (114). UDCA has immunomodulating actions and it reduces the expression of HLA (MCH) class 1 antigens in cholestasis (115).

Over 20 randomized placebo controlled studies evaluating the effect of UDCA on liver histology, laboratory values, survival, and liver transplantation exist. The Cochrane review evaluated 16 randomized studies (116). The positive effect was seen in liver biochemistry, the effect on liver histology was not clear, no benefit on mortality, although, the incidence of liver transplantations decreased. After the Cochrane analysis a combined analysis of four studies showed the benefit of UDCA on periportal necroinflammation, ductular proliferation, and stage progression in patients with early stages, I and II, of PBC, but no benefit could be shown if patients of all stages were regarded (117). The survival benefit is difficult to show in short treatment studies, therefore the meta-analysis from China (118) included only randomized studies over a two years duration with UDCA, a dose of 10 to 20 mg/kg/day. They found seven suitable trials and a meta-analysis of them showed a reduction of the need for liver transplantations and a marginal reduction in liver transplantations or death combined, but there were no effect on death alone with UDCA use. The latest (119) clinical review and meta-analysis of 16 randomized trials (1447 patients) found positive effect of UDCA only to bilirubin, ascites and jaundice, but they could not find significant benefit to mortality, mortality or liver transplantation combined, liver histology, pruritus, fatigue, autoimmune conditions, or to portal pressure. Short studies and the more severe stage of PBC were associated with a better effect of UDCA (119). These findings are controversial to the previous knowledge of the effect of UDCA.

Normalization of survival of patients with early stage PBC using UDCA was shown in a French study using multistate Markov model. The survival was equal compared to normal controls and significantly improved compared with predictive values of the Mayo risk score (120). This finding was confirmed with Dutch PBC patients using UDCA compared to Dutch controls. Patients with a normal serum albumin and bilirubin levels had similar life expectancy as the control population, but if albumin, bilirubin, or both were impaired, then the survival rates were decreased (121). Also in the Spanish study the patients with full biochemical response to UDCA had a similar prognosis as the control population. The survival was superior to the predictive Mayo score survival (122).

UDCA treatment is the only current medication for PBC accepted by the FDA. Its effect has been proved in the early stages of PBC (117), though current meta-analysis did not confirm this finding (119). UDCA, however, does not suppress inflammation for all of the patients. In 20 years, over 50% of the patients who were initially at stages I or II had progressed to stages III–IV or required a liver transplantation (120), at least those UDCA nonresponders are the patients who need combination therapies.



### 2.5.1.2 Prednisolone

The suppression of inflammation in the portal tracts with glucocorticoids is a tempting therapeutic possibility. Altogether three studies of PBC with prednisolone exist (Table 2) and in all of them a positive effect on liver histology was found (123–125), but the threat of bone loss has limited its use. In a study with prednisolone (30 mg/day tapered to 10 mg/day), the bone mass density (BMD) decreased almost twice as much as expected in one year (123). In a study with a longer duration the decrease in BMD was consistently greater up to three years in the prednisolone group than in the control group, but a statistically significant difference was only found after the first year (124). In Leuschners study (125), the BMD markedly decreased in one patient, in others the reduction was not significant.

**Table 2. Glucocorticoids in PBC.**

	Frankfurt, 9 months (125)		Newcastle, 1 year (123)		Newcastle, extension to 3 years (124)		Frankfurt, 2 years (126)	
	Prednisolone 10mg + UDCA	Control UDCA	Prednisolone 30mg-10mg	Control Placebo	Prednisolone (30mg)-10mg	Control Placebo	Budesonide 9mg +UDCA	Control UDCA
Number of patients	15	15	19	18	12	7	20	20
Death/transplantation	0	0	0	1	3	5	0	0
Histology improvement	7/15	0/14	6/19	0/17	4/9	0/7	30%	
Histology deterioration	1/15	5/14	1/19	6/17	1/9	4/7		3.5%
Portal hypertension	No data	No data	0	3/17	No data	No data	0	2/19
BMD(%)	1 decrease, others ns	ns	-4.2 (-10%)	0.7 (+2%)	ns	ns	-1.48 (-1.7%)	-0.41 (-0.98%)
Cirrhosis at 5 years	No data	No data	No data	No data	1/12	5/10	No data	No data

### 2.5.1.3 Budesonide

Budesonide is a non-halogenated glucocorticoid, and unlike prednisolone, about 90% of its oral dose is metabolized presystemically in healthy individuals (127). Budesonide is metabolized in the intestinal wall and liver by cytochrome P450 3A into two major metabolites, 16 $\alpha$ -hydroxy-prednisolone and 6 $\beta$ -hydroxy-budesonide. The glucocorticoid activity of these metabolites is only 1% to 10% of that of budesonide (127). Compared to prednisolone, the glucocorticoid receptor binding affinity of budesonide is 15 to 20 times higher, and it has been suggested that its effect on liver inflammation may be greater (128–130).

In PBC, budesonide 9 mg/day given together with UDCA for two years had a positive effect on liver histology in non-cirrhotic PBC patients without a significantly deleterious effect on the BMD of the lumbar spine (LS) (126)

(Table 2). In another study, which also included also patients with cirrhosis, budesonide 9 mg/day combined with UDCA had a significantly positive effect on bilirubin and alkaline phosphatase (ALP) levels, but resulted in a worsening of osteoporosis and hyperglycemia and an increase in the Mayo Risk Score, effects on histology were not evaluated (131).

#### **2.5.1.4 Other mediactions**

The immunosuppressive medications have been rather disappointing in PBC. One study of azathioprine in combination with prednisolone and UDCA exists. It was effective in one year of therapy in those patients who did not achieve full response to UDCA (132). Colchicine with its anti-inflammatory and antifibrotic effects has a slight additional effect in some patients when added to UDCA in early PBC (133), but not in advanced PBC (134). Meta-analyses of colchicine have not confirmed its effect (135). The UDCA combination with methotrexate was not effective (136). The other medications such as mycophenolate mofetil, cyclosporine, silymarin, simvastatin, sulindac, bezafibrate, and fenofibrate have mostly been studied in single studies with no proven efficacy to PBC (35).

### **2.5.2 Special features**

#### **2.5.2.1 Osteoporosis of PBC**

Osteoporosis is the primary metabolic bone disease in PBC, although the potential for osteomalacia exists. The metabolism of vitamin D is normal, but malabsorption of calcium and vitamin D may occur (137). The prevalence of osteoporosis and/or osteopenia in PBC are controversial. Some studies find the association with BMD and PBC and the severity of liver disease (138,139) and others only with age and postmenopausal status (137,140). The population cohort study from Nottingham found a 2-fold relative increase in any fracture in PBC patients compared with age and sex-matched control population (141).

The treatment of postmenopausal osteoporosis with estrogens is effective (142, 143) and had not resulted in worsening of cholestasis (142). Bisphosphonates, especially alendronate, are also effective on osteoporosis in PBC patients and their safety is good, (144,145) although long-term safety data does not exist.

#### **2.5.2.2 Pruritus**

The etiology of pruritus in PBC is unknown, but the symptom is common, 19% of patients already suffer from it at PBC presentation (2). Bile acids are suspected to mediate the pruritus in cholestasis, however, the plasma concentrations of bile acids do not correlate with pruritus. The possible substances circulating in the plasma in cholestasis, which could mediate pruritus, have not been found. Resins, cholestyramine and cholestipol may relieve pruritus, but they interfere with other medications and are not very

effective. Mechanisms of rifambicin are unknown, but it is helpful to some patients. The effects of antihistamines are weak and they only suit mild pruritus. (2,35).

Plasma separation and anion absorption decreases pruritus, but the effect is transient (146).

The opioidergic system and serotonin neurotransmitter system may be involved in pruritus on cholestasis. The opioid antagonists naloxone and naltrexone are effective in some patients (35). A recent study with serotonin uptake inhibitor sertraline, 75–100 mg/day, showed a marked improvement in pruritus compared with a placebo (147).

### **2.5.2.3 Fatigue**

Fatigue is the most common symptom at presentation of PBC (2) and it appears to be a very stable phenomenon (148). Fatigue in PBC is due to central nervous system (CNS) processes impaired by a combination of cholestasis and inflammation. Morphological abnormalities of the CNS are found secondary to accumulation of manganese (149). The impaired circadian rhythm, abnormal nighttime sleep, and sleepiness in daytime result to fatigue (150). Modafinil, a CNS-active agent, previously used for narcolepsia, obstructive sleep apnea, and shift-work sleep disorders, has shown to improve fatigue in PBC patients (151). The mechanisms of modafinil on fatigue may not only result from direct CNS effects, but also the effects on the autonomic nervous system and blood pressure regulation may be involved (152,153).

### **2.5.2.4 Hyperlipidemia**

The reduction of bile acid secretion in cholestasis leads to diminished bile acid synthesis and a down-regulation of hepatic cholesterol synthesis. The hepatic injury causes decline in functional LDL receptors and thus, the increase in total blood cholesterol, mainly LDL that has not been cleared by hepatocytes (154). The elevated LDL is mainly LP-X, an abnormal LDL particle, which has antiatherogenic properties and may reduce the atherosclerotic risk (155). In a study, where hypercholesterolemic PBC patients were compared with hypercholesterolemic non-PBC patients and normocholesterolemic patients, the risk for thickening of the intima in the carotic artery was only present in hypercholesterolemic non-PBC patients (156). The Dutch 14-year retrospective analysis demonstrated a 12% cardiovascular death rate in PBC patients (157). Thus, the need for cholesterol lowering agents depends on the other risk factors for atherosclerosis (155, 156). The intestinal cholesterol absorption is reduced in PBC and it is related to decreased bile acid synthesis, resulting in poor micellar solubilization (154). Therefore cholesterol absorption inhibitors, such as ezetimibe, may be an ineffective treatment for hypercholesterolemia. Statins as cholesterol lowering agents seem

to be safe when monitored carefully, though lovastatin, simvastatin, atorvastatin, and rosuvastatin are excreted in bile. In cholestasis statins may accumulate at toxic levels (155).

### **5.2.3 Liver transplantation**

In end stage liver disease the liver transplantation is a good option. In Finland, PBC has been the primary cause of liver transplantation, but recently the need for transplantations in other liver diseases has increased. The indications for liver transplantation in Finland are equal to other Nordic countries and consist of intractable pruritus or fatigue, relapsing bleeding from esophageal varices, refractory ascites or spontaneous bacterial peritonitis, hepatic encephalopathy, malnutrition, hepatocellular carcinoma, hepatopulmonary syndrome, or a serum bilirubin over 170  $\mu\text{mol/L}$  and serum albumin under 25 g/L. The survival after transplantation in PBC is among the highest of all causes of liver transplantation. Survival after liver transplantation at 1, 5, and 10 years was 82%, 75%, and 61% in the Birmingham transplantation unit (158). The recurrence of PBC in allograft was found histologically in 17% of patients after 3 years, but it did not affect the graft survival (158). In a meta-analysis, the recurrence of the PBC in the allograft was 18 % (159). No association was found to primary immunosuppression. In a German study the histological recurrence rate was 14% after 5 years and they found an association with recurrence to the use of tacrolimus as immunosuppressant (160). Two patients out of 14 with recurrence developed graft dysfunction. The survival in German patients at 5, 10, and 15 years was 87%, 84%, and 82%.

### 3 AIMS OF THE STUDY

The aims of the present study were to

1. examine the epidemiology of primary biliary cirrhosis in Finland and to evaluate whether the possible increase in prevalence was attributable to the increasing incidence, better survival, or both.
2. find out whether budesonide in combination with UDCA is an effective treatment on liver histology and laboratory markers of PBC compared with UDCA alone.
3. study the steady-state pharmacokinetics of budesonide and the bone mass density effects of 3-years of treatment with budesonide and UDCA, compared to UDCA alone, in patients with precirrhotic PBC and to relate the pharmacokinetics of budesonide to the stage of liver histology.
4. find out non-invasive serological markers to evaluate liver inflammation and fibrosis and the progression of PBC.

## 4 PATIENTS AND METHODS

### 4.1 Patients and methods in epidemiological study

#### 4.1.1 Case Definitions

The diagnosis of PBC was definite if all three of the following criteria were fulfilled: constantly elevated alkaline phosphatase, elevated antimitochondrial antibodies, and diagnostic or compatible liver histology.

Diagnosis was probable if only two of those three criteria were present. Incidence and prevalence rates include both definitive and probable cases.

#### 4.1.2 Date of Diagnosis

The date of diagnosis was the earliest date at which a patient fulfilled diagnostic criteria.

#### 4.1.3 Study Population

Patients were identified from four university hospitals (Helsinki, Turku, Tampere, and Oulu), their regional hospitals, and private medical centers in these areas. These areas cover southern, western, central, and northern parts of Finland (Study I, Figure1). The population of these study areas increased from 2 781 075 to 2 972 189 during the years 1988 to 1999, which represents 56.3% and 57.5% of the total Finnish population for these years.

Each Finnish resident has a unique personal identification code, which was used to locate their place of residence at the Population Information System maintained by the National Population Registry. The register system is updated online. Each patient's place of residence was confirmed annually (December, 31) during the study period from these registers.

#### 4.1.4 Study Period

The case finding period started in January 1, 1988 and ended in December 31, 1999. Incidence rates of new diagnosis of PBC per million inhabitants were calculated from January 1 to December 31 each year, and the prevalence rates for the annual mean population accordingly.

#### **4.1.5 Case Finding Methods**

Case finding methods: 1) A search for PBC was made from 25 hospitals discharge data regarding internal medicine or gastroenterology admission episodes, both the main diagnosis and additional diagnoses were recorded. Diagnosis codes 5716A, K74.3 (PBC) and 5716X, K74.5 (Biliary cirrhosis) were taken for further review. 2) A search from the archives of pathology departments for biliary cirrhosis. 3) Requests went to gastroenterologists in private clinics to identify PBC patients under their care. 4) A search of hospital discharge data was made in the Finnish Transplantation Unit, which serves all of Finland. The total amount of PBC liver transplantations from Finland are reported, as well as transplantations of the study population.

#### **4.1.6 Data Collection**

Hospital case records were reviewed in order to confirm the diagnosis, no data on disease severity or course was registered.

#### **4.1.7 Deaths**

The survival of patients until 31<sup>st</sup> of October 2004 was identified by record linkage of the study data with the National Causes of Death Register maintained by Statistics Finland, with date and underlying cause of death extracted from this register. Causes of death are coded according to the ICD-9 and ICD-10.

#### **4.1.8 Statistics**

Prevalence and incidence rates were expressed per million persons per year and age- standardized according to the direct method using the European standard population (65). Annual population counts for the denominators came from the National Population Information System, which is updated continuously. The trends in prevalence and incidence were determined by log-linear Poisson regression models with year as an independent variable. The regression coefficient of the year multiplied by 100 gives the average annual change in percentages. The 95% confidence intervals (CI) of the trend estimates were calculated from the standard error of the regression coefficient. Prevalence and incidence rates in the first and the last year of the study are reported as smoothed with the Poisson regression model to avoid the effects of random fluctuations. Factors related to survival after the PBC diagnosis were examined by Cox's proportional hazards regression analyses. Statistical analyses were carried out with SAS (SAS institute 1999). Ethical approval was obtained from the Ethics Committee of the Helsinki University Central Hospital.

## 4.2 Patients and methods in the treatment study

### 4.2.1 Patients

PBC stage I to III patients were collected from university hospital districts in three cities in Finland (Helsinki, Tampere, and Turku). A study information letter was sent to internists and gastroenterologists at local hospitals to recruit PBC patients to the trial. Consecutive patients were enrolled into the study, if inclusion and exclusion criteria were met. Before study entry and randomization, the diagnosis and severity of PBC was confirmed serologically (antimitochondrial antibodies S-AMA over 100 units (normal <50) and elevated serum alkaline phosphatase >300 U/l (normal <275 U/l)) and determined by a recent (within 12 months) liver biopsy. (Baseline data Table 3)

**Table 3. Baseline data of 77 randomized patients.**

	<b>Group A</b> <b>Budesonide + UDCA</b>	<b>Group B</b> <b>UDCA</b>
Number (male)	41 (5)	36 (4)
Age mean (range)	52.6 (33-67)	54.2 (25-70)
BMI kg/m <sup>2</sup> mean (range)	24.8 (18-38)	25.7 (20-30)
Previous treatment with UDCA	32	28
Stage I (%)	13 (17)	13 (17)
Stage II (%)	10 (13)	16 (21)
Stage III (%)	18 (23)	7 (9)
Grade 0	11	5
Grade I	14	15
Grade II	10	10
Grade III	6	6
Fibrosis 0	13	13
Fibrosis I	11	16
Fibrosis II	11	6
Fibrosis III	6	1

### 4.2.2 Exclusion criteria

Cirrhotic liver, stage IV

- Esophageal varices

- Portal or hepatic veins and arteries thrombosis, or reversed portal flow, or collaterals.

Age <18 or >70 years

Pregnancy or inadequate contraceptive use

Systemic use of corticosteroids or immunosuppressive medication

Clinically significant concomitant liver disease or positive hepatitis-B-surface-antigen (S-HBsAg), hepatitis-C-antibody (S-HCV-ab), smooth



muscle antibodies (SMA), and liver-kidney-microsomal antibodies (LKM).

4.2.3 Study protocol

The study design was randomized but open, because a placebo for budesonide was not available. The study endpoint was the change in liver histology. No washout period for patients receiving UDCA prior the study existed.

Randomization was done centrally at the Helsinki University Hospital with sealed envelopes in a block of ten and patients were stratified according to previous UDCA use, and stages I–II and III according to the Ludvig criteria (161). (Figure 3, Study flow chart)

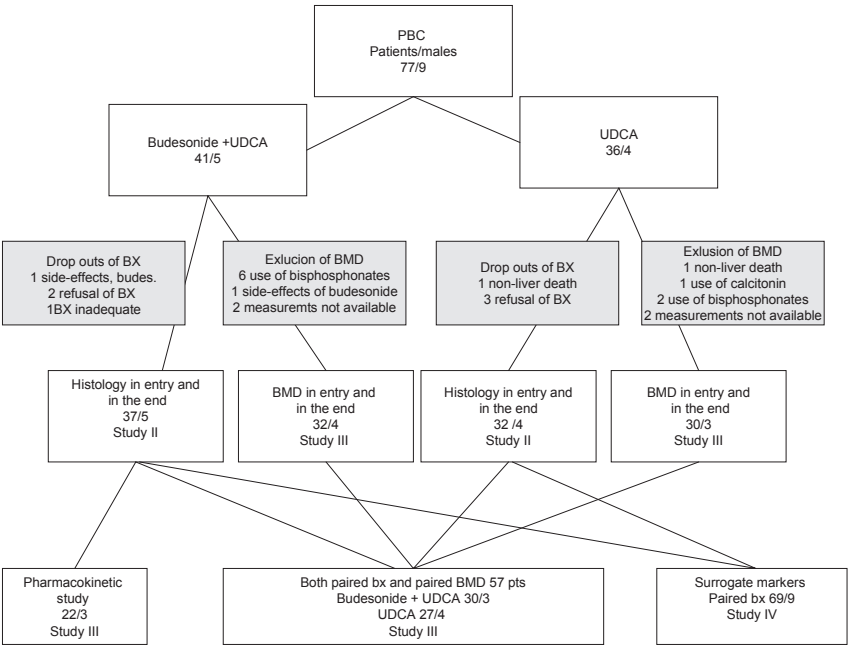


Figure 3. Study flow chart.

Upon study entry an esophago-gastro-duodenal endoscopy, Doppler ultrasound of the liver, bone mass density, liver biopsy (if not performed within 12 months), and a physical examination were performed and a complete medical history revealed.

At 4-month intervals, each patient had a physical examination and the following laboratory tests were measured: Alkaline phosphatase (ALP),  $\gamma$ -glutamyltranspeptidase (GT), alanine aminotransferase (ALT), bilirubin (bil), bile acids, albumin, prealbumin, prothrombin time, hemoglobin, leucocytes, platelets, erythrocyte sedimentation rate (ERS), plasma cortisol level, and fasting blood glucose (fb-gluc).

The following laboratory tests were made at 12-month intervals: amino-terminal propeptide of type III procollagen (S-PIIINP), vitamin-D (S-25(OH)D<sub>3</sub>), immunoglobulin M (IgM), and immunoglobulin G (IgG).

At the beginning and end of the study, serum-asetyltransferase (S-AST), s-hyaluronic acid (S-HA), urinary -N-telopeptide-collagen (U-NTX), plasma parathyroid hormone S-PTH, S-HbsAg, S-HCV-ab, S-AMA, S-SMA, S-LKM=antibodies, and antinuclear antibodies (S-ANA) were screened for.

After 3 years of therapy a liver biopsy, upper endoscopy, Doppler ultrasound of the liver were performed, BMD measured, and venous blood samples were collected for pharmacological measurements of budesonide.

#### **4.2.4 Study medication**

**Group A)** UDCA 15 mg/kg/day (divided into two doses; Adursal® 150 mg tablets, LeirasFinland, Finland) and budesonide 6 mg/day (single morning dose: 2 Entocort® 3 mg depot capsules, AstraZeneca, Finland)

**Group B)** UDCA 15 mg/kg/day.

#### **4.2.5 Other medications/ concomitant diseases**

All patients with inadequate dietary calcium or vitamin D intake were advised to use a supplementation therapy (calcium 1000 mg /day and vitamin D 400 units/day minimum), but the medication used was not controlled. Two patients were on thyroxin replacement therapy for primary hypothyreosis and were euthyreotic. None of the patients had medication for epilepsy, nor did any of them have parathyroid disturbances or other metabolic bone diseases. One patient with celiac disease was on a gluten-free diet.

#### **4.2.6 Histological evaluation**

Liver biopsies were evaluated by a single pathologist, who was blinded to the clinical data and biopsy sequence. Ludwig criteria (161) was used to analyze the stage (I = portal hepatitis, II = periportal hepatitis, III = bridging necrosis or fibrosis or both, IV = cirrhosis). The METAVIR point score (162) to evaluate inflammation activity from 0 to 3, which is based on lobular and interface inflammation. Lobular inflammation is

graded: 0 = less than one focus, 1 = one focus per lobule, and 2 = multiple foci per lobule or bridging necrosis. Interface inflammation or lymphocytic piecemeal necrosis is graded: 1 = focal in some portal areas, 2 = focal in most portal areas or diffuse in some, 3 = diffuse in all portal areas.

The METAVIR point score (162) was also used to assess fibrosis: 0 = normal, 1 = portal expansion, 2 = porto-portal septa formation, 3 = porto-central septa formation, and 4 = cirrhosis. Stainings were at least hematoxylin-eosin for inflammation and van Gieson or Herovici for fibrosis.

#### **4.2.7 Bone mass density**

The BMDs were examined by dual-energy absorptiometry (DEXA) densitometers, i.e. with Lunar Prodigy (GE Lunar Corporation, Madison, USA) in the Tampere University Central Hospital, with Hologic QDR-1000 (Waltham, MA, USA) in the Helsinki University Central Hospital, and with Hologic QDR-4500C in the Turku University Central Hospital. BMD values were obtained from the lumbar vertebrae L1-L4 and the femoral neck. The T-score values at baseline, and the individual changes in BMD from the baseline to the study end were used in statistical analysis.

#### **4.2.8 Questionnaire**

All patients completed a questionnaire containing 41 questions about lifestyle factors affecting bone mass density and 11 additional questions for women concerning hormonal factors and medications. Coffee and alcohol use, smoking, exercise, use of calcium and vitamin-D supplementation, diuretics, herbal medication, use of corticosteroids and anabolic steroids, fractures, other diseases and their treatments, use of milk products, exposure to sunlight, and for women time of menarche and menopause, contraception, pregnancies, ovarian operation, and estrogen replacement therapy were covered.

#### **4.2.9 Pharmacokinetic measurements of budesonide**

After an overnight fast, a single dose of 6 mg budesonide (2 Entocort® 3 mg depot capsules, AstraZeneca, Finland) was ingested at 8 am, and venous blood samples were collected at 0, 3, 6, 8, 10, 16, and 24 hours postdose into tubes containing ethylenediaminetetraacetic acid (EDTA). The patients fasted for at least four hours after the administration of budesonide. After sampling, plasma was separated and stored at -80°C until analysis. Plasma budesonide and cortisol concentrations were quantified by liquid chromatography-tandem mass spectrometry, as described earlier (163). The quantification limit for budesonide was 0.05 ng/mL and the day-to-day coefficient of variation (CV) was 11% at 0.05 ng/mL, 6.4% at 0.4 ng/mL, and 3.6% at 2.0 ng/mL (n=5). The quantification limit for cortisol was 0.5 ng/mL, and the CV was 3.9% at 4.0 ng/mL, 5.9% at 24 ng/mL, and 2.5% at 120 ng/mL (n=5).

The pharmacokinetics of budesonide were characterized by the peak concentration ( $C_{\max}$ ) in plasma, the time to  $C_{\max}$  ( $t_{\max}$ ), the area under the concentration-time curve from 0 to 24 hours [ $AUC(0-24h)$ ], and elimination half-life ( $t_{1/2}$ ). The  $C_{\max}$  and  $t_{\max}$  values were taken directly from the original data. The terminal log-linear part of each concentration-time curve was identified visually, and the elimination rate constant ( $k_e$ ) was determined from the log-transformed data using the linear regression analysis. The  $t_{1/2}$  was calculated by the equation  $t_{1/2} = \ln 2/k_e$ . The  $AUC(0-24h)$  values were calculated by use of the linear trapezoidal rule. All pharmacokinetic calculations were performed with the program MK-Model, version 5.0 (Biosoft, Cambridge, UK).

#### 4.2.10 Biochemical assessment

Serum concentrations of ALP, GT, ALT, AST, BIL, albumin, prealbumin, prothrombin time, hemoglobin, leucocytes, platelets, ERS, plasma cortisol level, and galactose elimination test were measured by standard laboratory methods. NTX excretion, osteocalcin, PTH, total cholesterol and triglycerides, and FB-GLUG were taken after an overnight fast. The NTX was measured in urine (fresh morning urine) by an enzyme-linked luminoimmunoassay, the PIIINP was measured by a radioimmunological assay from Orion Diagnostica (Espoo, Finland), HA by an enzyme-linked binding protein assay (Corgenix, Tejon St. Westminster, USA), osteocalcin in serum by immunoradiometric assay, and PTH in plasma by immunochemiluminometric assay in a quality controlled laboratory. Total cholesterol and noncholesterol sterols in serum were measured from nonsaponifiable material by gas liquid chromatography (164,165). Noncholesterol sterols include cholestanol, cholesterol precursor sterols:  $\Delta 8$ -cholestenol, lathosterol, and desmosterol, and two plant sterols: campesterol and sitosterol. The data are expressed as mmol/mol of cholesterol ( $\times 10^2$ ).

#### 4.2.11 Non-invasive scores

Various non-invasive scores derived from the laboratory data were calculated according to previously established criteria as follows:

Forn's score =  $7.811 - 3.131 \cdot \ln(\text{platelet count } 109/l) + 0.781 \cdot \ln(\gamma\text{GT U/l}) + 3.467 \cdot \ln(\text{age, y}) - 0.014 \cdot (\text{cholesterol, g/l})$  (101).

AST/platelet ratio index (APRI) =  $\text{AST/ULN} \cdot 100 / \text{platelet count, } 109/l$  (102).

Fibrosis index =  $\text{S-bilirubin } [\mu\text{mol/l}] / 14 + \text{HA} [\mu\text{g/l}] / 143$  (166).

PBC score =  $\text{HA} [\mu\text{g/l}] + \text{PIIINP} [\mu\text{g/l}] + \text{AST } [\text{U/l}] + \text{bile acids } [\text{mmol/l}]$ ,

$\text{LnPBC-score} = \ln(\text{AST}) + \ln(\text{HA}) + \ln(\text{PIIINP}) + \ln(\text{BA})$ . (PBC scores are not validated in literature before).

#### **4.2.12 Statistics**

The primary end point was improvement in liver histology. The sample size calculation was based on an assumption that 30% improvement will be seen at liver histology in the combination group compared to UDCA alone. Using the  $\alpha$  of 0.05 and 80% power a sample size of 80 patients allows sufficient power to detect a 30% improvement in liver histology. All data are expressed as mean  $\pm$  SD. For comparison, the Student's *t*-test and Mann-Whitney U-test were used. When variances were unequal, or their distribution was not normal, the Kruskal-Wallis multiple comparison test was used. A comparison of incidences was performed with  $\chi^2$  statistics or Fisher's exact test, and associations between the variables were tested by using the Pearson's correlation coefficient. Statistical calculations were performed with NCSS-2000 software for Windows (NCSS Statistical Software, Kaysville, UT) or with SPSS 13 software for Windows (SPSS, Inc, Chicago, IL, USA).

The study was approved by the Ethics Committee of the Helsinki University Hospital and the National Agency for Medicines. All patients gave their informed consent for participation.

## 5 RESULTS

### 5.1 Epidemiological study

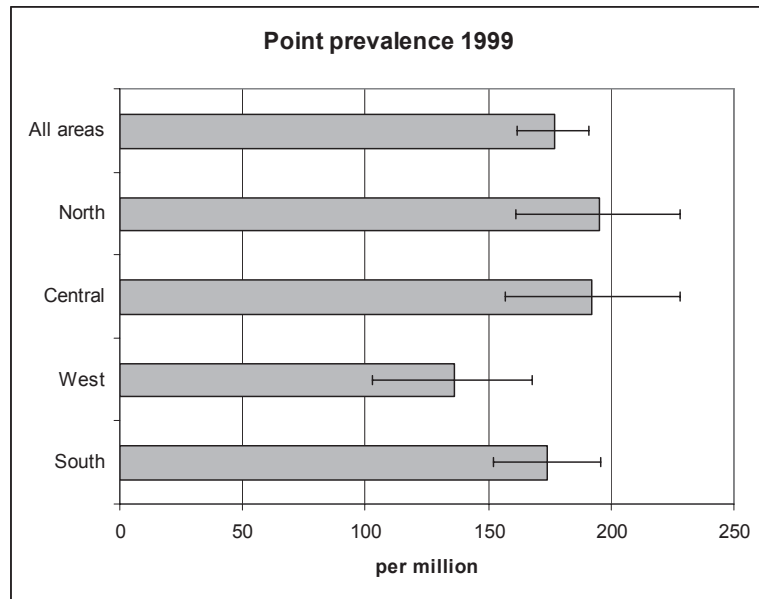
#### 5.1.1 Study population description

During the study period (1988–1999), the PBC population increased from 294 to 545 patients. The characteristics of them are described in Study I, Table 1. The proportion of women was rather stable, 85% to 87%. The diagnosis was definite for approximately 78% of the patients. The 22% of probable PBC diagnoses comprised AMA-negative patients (11%), those not confirmed by liver biopsy (9%), and those with normal ALP (2%).

Median age at diagnosis was 58 years in the beginning of the study and diminished a few years during the study period, but not significantly, while the median age at death increased significantly from 65 to 76 years ( $p = 0.001$ ). The median time from diagnosis to death increased from 6 to 11 years ( $p = 0.034$ ) already after the first three years and stayed stable thereafter.

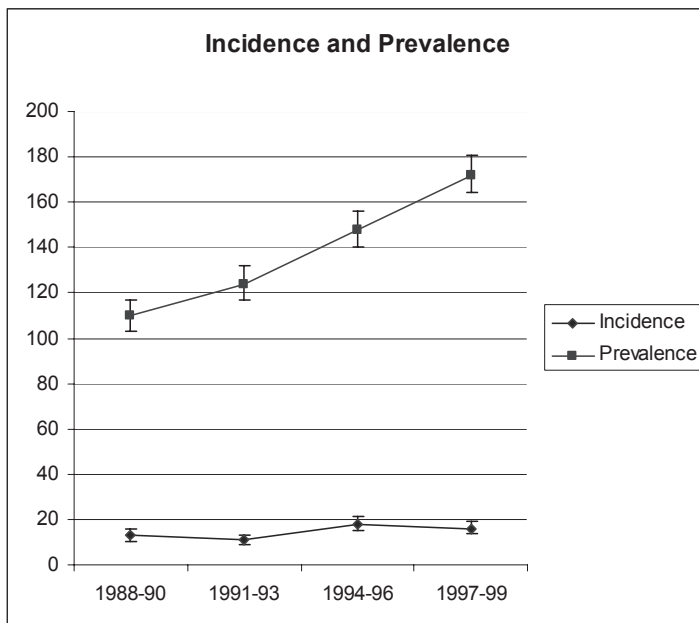
#### 5.1.2 Prevalence

In the beginning of the study, the overall age-standardized prevalence of PBC was 103 (95% CI 97 to 110) per million inhabitants, increasing to 180 per million (172–189) by study end. In sex-specific analyses, the age-standardized prevalence in women was 161 (151–171) and increased to 292 (277–307) per million. In men, the prevalence increased from 33 (28–38) to 55 (48–63).



**Figure 4.** Point prevalence of PBC in 1999 in Finland (95% confidence interval).

The point prevalence in 1999 in geographical areas is presented in Figure 4. The prevalence tended to be lower in western Finland compared with the other areas, for both women and men. The annual average increase in prevalence in the whole study area was 5.1% ( $p < 0.0001$ ); in the South 5.1%, in the North 6.8%, in the Central Finland 4.1% ( $p < 0.0001$ ), and in the West 3.7% ( $p = 0.001$ ). Prevalences are presented in three year periods in Figure 5.

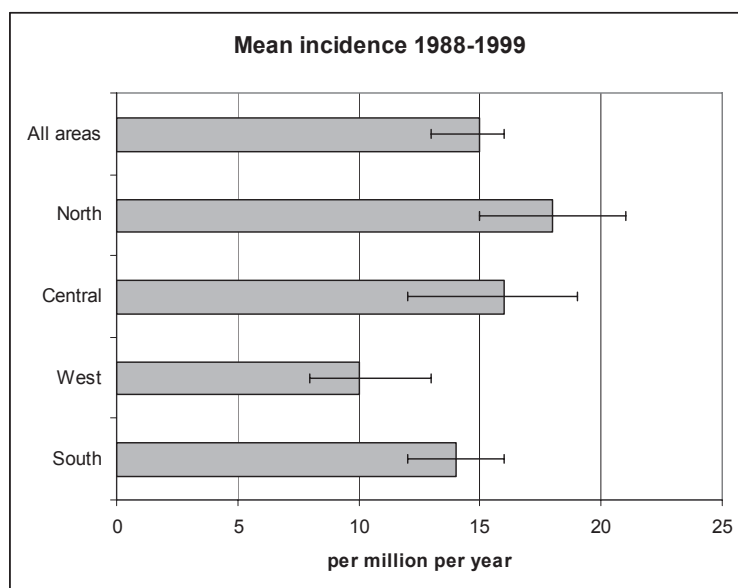


**Figure 5.** The annual average incidence and prevalence of three year periods in the study area with 95% confidence intervals. Incidence is presented per million per year and prevalence per million inhabitants.

### 5.1.3 Incidence

The mean age-standardized average incidence rates during the whole study period are presented by area in Figure 6. In western Finland the incidence tended to be lower than in other areas. When data on both sexes were combined, the difference between west and north became significant, but otherwise no differences appeared between the study areas. During the study period the age-standardized incidence increased from 12 (10–14) to 17 (15–20) per million annually. In women, the incidence increased from 20 (16–24) to 27 (23–32) and in men from 3 (2–5) to 8 (6–11). The annual average increase in incidence in the whole study area was 3.5% ( $p = 0.008$ ): in the South 4.2% ( $p = 0.04$ ), the North 1.7%, the Central 3.2% and the West 5.2%, ( $p = ns$ ). The annual average incidence in three year periods is presented in Figure 5.





**Figure 6.** Mean age-standardized incidence of PBC in Finland 1988–1999(95% confidence interval).

#### 5.1.4 Mortality

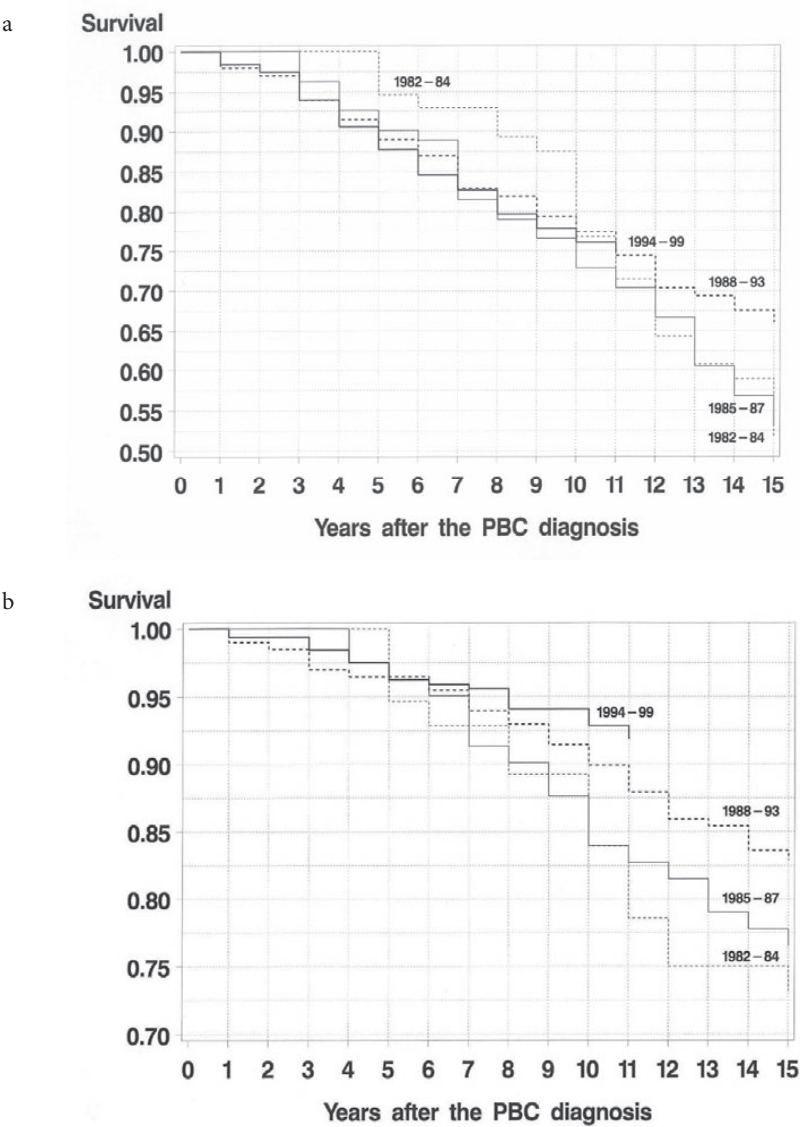
During the study period (1988–1999) a total of 185 PBC patients died in the study area, over half (98) of them died of liver-related causes, including seven hepatocellular carcinomas (HCC), other causes consisted of other malignancies (18), coronary heart disease (21), micellaneous causes (48). During the follow-up until 2004, 124 additional patients died, 54 of them for liver-related causes (10 HCC), other malignancies (11), coronary heart disease (16), and micellaneous causes (43). Death and transplantation rates per year and per PBC patient population are presented in Study I, Ttable 1. Approximately 4.2% of the patients died annually.

#### 5.1.5 Survival

The age, sex, and study area adjusted hazard ratio (HR) for all-cause deaths was 0.6 (0.4–0.9,  $p = 0.01$ ) per 10-year increment in time, and HR for liver-related deaths was 0.4 (0.2–0.8,  $p = 0.006$ ) per 10-year increment. Survival in the four timepoints (1982–1999) of diagnosis for all-cause deaths and for liver-related deaths are shown in Figures 7a and b. Survival of this PBC population improved during the study years and follow-up period.

No significant survival differences occurred between the study areas. Increasing age and male sex were significantly associated with increasing risk of death: HR = 2.5 (2.1–2.9,  $p < 0.0001$ ) per 10-years increment in age and HR = 1.6 (1.1–2.2,  $p = 0.009$ ) for male sex. When liver-related deaths were used as the outcome, increasing age and early calendar time of the

PBC diagnosis significantly increased the risk of death: HR = 2.1 (1.7–2.7,  $p < 0.0001$ ) and HR = 0.4 (0.2– 0.8,  $p = 0.006$ ) per 10-year increment in age or in time of PBC diagnosis.



**Figure 7** a,b. Survival in PBC in four time points of diagnosis, a) all deaths, b) liver related deaths.

### 5.1.6 Liver transplantations

In all of Finland, 546 patient received their first liver transplantation between 1990 and 2006, and 85 (15.6%) of them were PBC patients. The relative amount of transplantations due to PBC decreased over time. In five-year-periods 1990–1994, 1995–1999, and 2000–2004 the PBC transplantations represented 25%, 19%, and 11% of all first liver transplantations. From the study population 37 patients were grafted for PBC during the study period 1988–1999 and four patients before that (1985–1987). The overall liver transplantation rate from this PBC population was 5.4%.

## 5.2 Treatment study

### 5.2.1 Study population description

The baseline characteristics of 77 randomized patients appear in Table 3. The factors associated with osteoporosis, menopause, estrogen replacement therapy, smoking, and alcohol consumption, did not differ between the groups. All patients were HBsAg and HVC-antibody negative. ANA positivity was found in approximately 7% of patients.

**Table 3. Baseline data of 77 randomized patients.**

	<b>Group A</b> <b>Budesonide + UDCA</b>	<b>Group B</b> <b>UDCA</b>
Number (male)	41 (5)	36 (4)
Age mean (range)	52.6 (33-67)	54.2 (25-70)
BMI kg/m <sup>2</sup> mean (range)	24.8 (18-38)	25.7 (20-30)
Previous treatment with UDCA	32	28
Stage I (%)	13 (17)	13 (17)
Stage II (%)	10 (13)	16 (21)
Stage III (%)	18 (23)	7 (9)
Grade 0	11	5
Grade I	14	15
Grade II	10	10
Grade III	6	6
Fibrosis 0	13	13
Fibrosis I	11	16
Fibrosis II	11	6
Fibrosis III	6	1

## 5.2.2 Biochemical markers

**Table 4. Laboratory values at baseline and after 36 months.**

Variables mean (±SD)	Budesonide + UDCA (Group A)			UDCA (Group B)			Change between groups <i>p</i> value
	0 months	36 months	<i>p</i> value	0 months	36 months	<i>p</i> value	
S-ALP, U/L	490 (313)	345 (210)	0.0002	516 (343)	388 (220)	0.02	ns
S-Alt, U/L	64 (50)	40 (25)	0.0003	61 (40)	45 (25)	0.02	ns
S-GT, U/L	203 (203)	107 (133)	0.0005	242 (297)	144 (296)	0.02	ns
S-AST, U/L	41.7 (46.0)	27.6 (15.9)	<0.0001	46.0 (41.7)	31.7 (14.1)	0.03	0.048
S-Alb, g/L	41.3 (2.9)	40.9 (2.3)	ns	41.7 (2.7)	42.5 (2.8)	ns	0.04
S-Prealb mg/L	235 (49)	229 (57.5)	ns	237 (41)	227 (42.3)	0.01	ns
P-Prothrombin time, %	122.5 (26)	109.5 (45.5)	ns	127.2 (28)	111.7 (36.4)	ns	ns
B-Platelets, E <sup>9</sup> /L	264 (56)	262 (66)	ns	267 (108)	253 (94)	ns	ns
S-Bil, µmol/L	12.2 (4.7)	11.2 (4.0)	ns	10.8 (4.4)	12.4 (4.8)	0.1	0.002
S-IgM, g/L	3.4 (2.0)	2.3 (1.3)	<0.0001	4.2 (2.6)	3.4 (2.5)	0.02	ns
S-IgG, g/L	14.0 (3.3)	11.1 (2.1)	<0.0001	13.4 (3.3)	12.5 (2.4)	0.01	0.0007
B-ESR, mm/h	35 (21)	22 (14)	<0.0001	32 (20)	29 (16)	ns	0.02
S-PIIINP, µg/L	4.6 (1.7)	3.1 (1.5)	<0.0001	4.2 (1.3)	3.6 (1.6)	0.02	0.01
U-NTX, nmol/mmol creatinine	50.8 (38.4)	43.6 (21.3)	ns	40.1 (20.6)	29.6 (15.2)	0.007	ns
S-HA, ng/ml	38.8 (30.5)	38.3 (30.3)	ns	32.9 (21.1)	41.4 (34.3)	ns	ns
Galactose elimination test, min	13 (6.4)	14 (5.0)	ns	16 (4.7)	16 (6.5)	ns	ns

Values are presented as means (±SD);  $p \leq 0.05$  considered significant, ns : non significant.

The values for ALP, ALT, GT, and AST improved in both groups (Table 4). A significant statistical difference between groups appeared for bilirubin, AST, Alb, IgG, ESR, and PIIINP. Mean bilirubin levels were within normal limits in both groups, the levels stayed stable in group A ( $p = ns$ ) and rose in group B ( $p = 0.01$ ). The mean albumin and prealbumin levels remained unchanged in group A, whereas in group B the mean albumin rose ( $p = ns$ ) and prealbumin decreased ( $p = 0.01$ ), however, they remained within the normal range. S-IgG, ESR, and also a marker for fibrosis, PIIINP, decreased significantly in group A. U-NTX showed no difference between groups, suggesting that bone resorption was not increased in group A. The only difference in biochemical markers of bone metabolism between the study groups (osteocalcin, PTH, Calcium, 25(OH)D<sub>3</sub>) was a significant increase in vitamin-D in the group B ( $p < 0.05$ ) (Study III, table 4). The galactose elimination test, prothrombin time, and platelets remained stable in both groups, which were expected in non cirrhotic PBC. The mean levels of plasma cortisol decreased after 2 years in group A, demonstrating a slight systemic effect for budesonide (Study II, Figure 1). Glucose values did not rise compared to group B levels (Study II, Figure 1).

### 5.2.3 Doppler ultrasound and gastroscopy

No changes were detected at 36 months in the Doppler ultrasound or by upper endoscopy in any patient compared with baseline, except the one who developed hypocortisolism. She was dropped out of the study at 20 months. The Doppler ultrasound demonstrated some minimal changes in her liver with no collaterals, but gastroscopy revealed grade-one varices (Study II).

### 5.2.4 Liver histology

Sufficient paired liver biopsies for analysis were available from 69 patients: 37 patients in the combination group (A) and 32 in the UDCA group (B). In surrogate analysis the groups were not separated. Of liver biopsies 96% contained either >6 portal tracts or the length was  $\geq 10$ mm. At baseline, more patients who were randomized into combination therapy already had stage III disease, 18 vs 7 at randomization and 15 vs 6 at analysis ( $p = 0.05$ ).

The improvement in liver histology was seen in group A in the stage (22%,  $p = 0.06$ ), fibrosis (25%,  $p = 0.08$ ), and grade (34%,  $p = 0.02$ ), while the deterioration of stage (20%,  $p = 0.07$ ) and fibrosis (70%,  $p = 0.005$ ) occurred in group B. The grade improved insignificantly in group B by 10%. The significant differences between the groups were found in the stage ( $p = 0.009$ ) and fibrosis ( $p = 0.0009$ ) in favor for the combination group (A), but not in the grade which was improved in both groups. The changes in grade consist of lobular hepatitis and interface hepatitis. The interface hepatitis improved in group A 42% ( $p = 0.006$ ) and 8% ( $p = \text{ns}$ ) in group B, no statistical difference between the groups appeared. The lobular hepatitis improved by 25% ( $p = \text{ns}$ ) in group A and deteriorated by 17% in group B ( $p = \text{ns}$ ), no statistical difference existed between the groups.

The change in stage according to the baseline stage stratification (Table 5) demonstrates the statistically significant difference between the treatment arms only at stage II and at stages I and II combined. The histology in 8 patients in the combination group improved towards normal, while no one reached normal histology in UDCA group (Table 5). Analyzing the stage by means of improvement/no change/worsening, the significant positive effect in the combination group, compared with the UDCA group, is found in stages I and II separately and in all stages combined, but not in stage III separately. When the effect of the degree of grade on change of stage was analyzed, significant differences between groups were found: if the grade was  $\geq 2$ , the stage improved significantly in group A compared to UDCA alone,  $-0.67$  vs  $0.27$ ,  $p < 0.01$ . If the grade was  $\leq 1$ , the difference of the delta stage did not reach statistically a significant difference ( $-0.27$  vs  $0.4$  in group A and B).

**Table 5. Initial stage and consecutive stage after treatment in the combination group and in the UDCA group. When initial stages I and II are combined  $p = 0.04$ .**

Initial stage	Consecutive stage							<i>p</i>
	Budesonide and UDCA				UDCA			
	0	I	II	III	I	II	III	
I	2	5	5	0	3	6	2	ns
II	4	2	2	2	5	3	7	0.04
III	2	1	5	7	0	2	4	ns

### 5.2.5 Pharmacokinetics of budesonide

Pharmacological measurements were analyzed from 22 patients of whom 3 were in histological stage 0, 5 in stage I, 7 in stage II, and 7 in stage III at the time of analysis. The  $C_{max}$ , AUC(0–24h), and  $t_{1/2}$  of budesonide did not differ significantly between the stages 0 and I combined, II, and III, Study III (Study III, Table 3). In those three patients with stage 0 the AUC(0–24h) seemed to be lower than in stages I–III, (Study III, Figure 1), but statistical analysis was not appropriate due to the small number of subjects in stage 0. No statistically significant correlations between the  $C_{max}$ , AUC(0–24h) or  $t_{1/2}$  of budesonide, and change of histological stage or grade, bilirubin level, or change of BMD were found.

### 5.2.6 Effects on bone

Analysis of the bone mass density (BMD) is based on 57 patients who did not receive bisphosphonates during the study and both paired liver histology and BMD measurements were available.

The BMD in the baseline was slightly reduced in both groups compared with the normal population: the T-score in the femoral neck (FN) was -0.92 (SD 0.8) in group A and -0.94 (1.1) in group B and the T-score in the lumbar spine (LS) was -0.81 (0.96) and -0.76 (1.36). The mean BMD in the FN decreased during 3 years by 3.6% in group A ( $p = 0.0002$ ) and by 1.9% in group B ( $p = 0.029$ ). The reduction in BMD of the LS was 2.8% ( $p = 0.003$ ) in group A and 0.7 % ( $p = 0.25$ ) in group B. The difference in the reduction of BMD between groups, however, did not reach statistical significance in FN ( $p = 0.16$ ) or in LS ( $p = 0.08$ ), (Study III, Figure 2). The changes of BMD in patients using budesonide did not correlate to the initial BMD, stage of liver disease, or tobacco smoking.

### 5.2.7 Adverse events

Two patients had major glucocorticoid dependent side-effects: in one patient the cortisol value decreased and the budesonide therapy was stopped (Study flow-chart figure 3), another patient had low cortisol values which normalized by reduction of the budesonide dose to 3 mg/day. Seven

patients reported mild glucocorticoid related side effects: bruises, thinning of skin, acne, nausea, mild hirsutism, and some weight gain (3 patients). None of these symptoms were severe requiring neither change in treatment nor in budesonide dose. The majority of the side-effects appeared in patients with stage III PBC (five compared to two at earlier stages). In group B, two patients had itching related to elevation of UDCA dose.

### 5.2.8 Biochemical markers and histology

Bilirubin and albumin levels were equal and normal in all stages. The AST, bile acids, PIIINP, and HA were statistically different between the stages at baseline and at 36 months (except bile acids). Cholesterol precursors, cholestanol, campesterol, and sitosterol or their ratio did not differ between the stages.

The PBC-score, combination of HA, PIIINP, bile acids, and AST demonstrated the best correlation with the different stages ( $p < 0.0001$ ), Table 6. Logarithmic(ln) transformation of individual variables of the score improved discrimination value of ANOVA,  $p$ -value for the lnPBC-score was 0.00002. The discriminant function for variables of lnPBC-score for stage is:  $-1.56 \cdot \ln(\text{AST}) - 0.71 \cdot \ln(\text{HA}) - 0.10 \cdot \ln(\text{PIIINP}) - 0.55 \cdot \ln(\text{BA}) + 7.85$ ,  $p = 0.009$ . Of other calculated scores only the AST/platelet ratio index (APRI) demonstrated a significant association between the stages both at baseline and after 36 months of therapy. The ALT/AST-ratio did not detect different stages, neither could the Forn's index nor Fibrosis index differentiate the stages at baseline.

The grade of lymphocytic piecemeal necrosis (LPN) was classified as dichotomic variable as follows: none to mild inflammation (0–1) and moderate to severe (2–3). At baseline the serum levels of ALP, IgG, HA, and PIIINP demonstrated a significant difference between mild and severe LPN. Only PIIINP was constantly associated with the degree of LPN. The scores for APRI, ALT/platelet, PBC-score, and campe/sito-ratio were statistically different between groups at baseline. The discriminant function for variables of lnPBC-score for LPN is:  $1.21 \cdot \ln(\text{AST}) + 0.56 \cdot \ln(\text{HA}) + 0.74 \cdot \ln(\text{PIIINP}) + 0.26 \cdot \ln(\text{BA}) - 7.81$ ,  $p = 0.020$ .

Fibrosis was scored to none or minimal fibrosis (F0-1) or increased fibrosis (F2-3). Of the laboratory parameters only AST, HA, and PIIINP were significantly associated with degree of fibrosis, both at baseline and at 36 months. From the scores, only APRI and PBC-score were significantly different between F0-1 and F2-3. The discriminant function for variables of lnPBC-score for fibrosis is:  $0.69 \cdot \ln(\text{AST}) + 0.24 \cdot \ln(\text{HA}) + 1.53 \cdot \ln(\text{PIIINP}) + 0.59 \cdot \ln(\text{BA}) - 6.77$ ,  $p = 0.026$ .

We were unable to find any association between the changes of HA, PIIINP, bile acids, or their sum (PBC-score), and the change of the stage, degree of fibrosis, or grade. Only the changes in Forn's score were significantly different (ANOVA) in patients with improvement or no

change in stage compared to those with worsening of the stage and the change of campe- and sitosterol revealed association with the change of fibrosis graded accordingly (data not shown).

**Table 6. The P value of biochemical markers and serological scores according to the histological stage, lymphocytic piecemeal necrosis (LPN), and fibrosis (F) at baseline and at 36 months**

Variable	Stage 0-1 vs 2 vs3		LPN 0-1 vs LPN 2-3		F 0-1 vs F 2-3	
	Baseline	36 mo	Baseline	36 mo	Baseline	36 months
B-platelets	NS	NS	NS	NS	NS	NS
S-bilirubin	NS	NS	NS	NS	NS	NS
S-AST	<0.05	<0.05	NS	NS	<0.05	<0.05
S-ALT	NS	<0.05	NS	NS	NS	<0.05
S-ALP	NS	<0.05	<0.05	NS	NS	NS
S-gGT	NS	NS	<0.01	NS	NS	<0.05
S-IgM	NS	NS	<0.05	<0.01	NS	NS
S-IgG	NS	NS	NS	<0.05	NS	<0.01
S-bile acids	<0.05	NS	NS	NS	<0.05	NS
S-HA	<0.05	<0.01	<0.05	NS	<0.01	<0.01
S-PIIINP	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05
S-Cholesterol	NS	NS	NS	NS	NS	NS
S-Cholestanol,	NS	0.05	NS	NS	NS	<0.05
S-Sitosterol	NS	NS	NS	NS	NS	NS
S-Campesterol	NS	NS	NS	NS	NS	NS
APRI	<0.01	<0.05	<0.05	NS	<0.05	<0.05
ALT/platelet	NS	<0.05	<0.05	NS	NS	<0.05
AST/ ALT	NS	NS	NS	NS	NS	NS
Fibrosis index	NS	<0.05	NS	NS	NS	<0.05
Forn's	NS	NS	NS	NS	NS	NS
PBC-score	<0.0001	<0.01	<0.01	NS	<0.001	<0.01
Campe/sito	NS	NS	<0.05	NS	NS	NS

### 5.2.9 Sensitivity and specificity of biochemical markers

The correlation of HA with stage and the METAVIR fibrosis score at baseline was  $r_s = 0.356$  and  $r_f = 0.322$ . The respective correlations for PIIINP were  $r_s = 0.384$  and  $r_f = 0.410$ , for APRI  $r_s = 0.338$  and  $r_f = 0.262$ , for PBC-score  $r_s = 0.513$ ,  $r_f = 0.481$ . Areas under the ROC curves (AUROC) were used to evaluate the sensitivity and specificity of biochemical parameters and the calculated scores. The PBC-score demonstrated the highest value for AUROC for stage 0–1 vs stage 2–3, both at baseline and after 36 months. AUROC was 0.785 for the PBC-score, 0.673 for HA, 0.660 for PIIINP, and 0.720 for AST. Using a cut-off value of 66 for the PBC-score the sensitivity was 81.4% and specificity 65.2% for classifying the stage of PBC. Dropping the cut-off value to 57 the sensitivity increased to 86.0% and specificity dropped to 40.0 %. For fibrosis, all the biochemical variables and calculated scores were less sensitive. S-AST and the PBC-score were the most sensitive parameters to evaluate the grade of LPN.



## 6 DISCUSSION

### 6.1 Epidemiology of PBC

The prevalence and incidence of PBC are increasing in the western world. Almost every prevalence data from North America or Europe collected after 1994 are higher than data collected before that year (Figure 1). The same trend is also seen in the incidence, though not as clear as in prevalence. The USA (52) and UK (54) are the most prevalent places of PBC in the world and high numbers from the UK were already reported at 1980s. Our data showed a marked annual increase both in prevalence (5.1%) and in incidence (3.5%) over 12 years time period in Finland (Study I). The Finnish epidemiological data are comparable to the Swedish (49,50,55) and Norwegian ones (56), but Nordic prevalence and incidence numbers are still far from the USA and UK. The differences within geographical areas studied in Finland were small, though the western Finland showed a lower prevalence than the rest of Finland (Figure 4). The incidence in the west was also lower than in Northern Finland, but not different compared with the other parts of Finland (Figure 6). The reasons for these lower numbers in western Finland are difficult to find, the health care system should be equal in different parts of Finland. One possible explanation for the higher prevalence in the north is the migration of young women to the southern parts of Finland, leaving the rapidly increasing prevalence in the north while new diagnosis of PBC are made more in the southern parts of Finland leading to the increasing incidence in the south.

Some of the increase might be explained by better awareness of the disease and active use of laboratory tests in occupational health care in non-symptomatic patients. This, however, can not explain everything. The age of the patients having a new diagnosis did not markedly decrease in 12 years in the study population. Mitochondrial antibodies are very specific for PBC and have already been used for four decades, so the diagnosis is easy to make with biochemistry and confirmation with histology is optional. In our study (I), we accepted both defined and probable diagnosis to prevalence and incidence numbers, as have also other surveys done, and the proportion of definite diagnosis during the study period was stable (in three years periods 80%, 78%, 80%, 80%), which supports the idea that an enhanced diagnostic work up is not the main reason for the increased prevalence and incidence of the PBC.

The improved overall survival was also confirmed in the Finnish population: 40% survival improvement in all deaths and 60% for liver related deaths per 10-year time increment. This obviously explains part of the increasing prevalence. Also, the excellent results of liver transplantations decrease the mortality of these patients. In Finland, at 1, 5, and 10 years after transplantation the PBC survival was approximately 95%, 88%, and 77% (Personal information. Isoniemi H, 2007). For comparison the survival in Birmingham transplantation unit was 82%, 75%, and 61% (158). The transplantation itself does not cure PBC and recent publications show that the recurrence of PBC in liver allograft occurs in approximately 18% of the patients (159). The recurrence of disease seldom causes loss of the allograft.

Environmental factors may explain the increase in PBC incidence. *Novosfingobium aromaticivorans*, a gram negative bacterium, which has the highest level of homology between any known microorganism and human PDC-E2, can metabolize xenobiotics and give a start to the autoimmune process leading to inflammation and destruction of bile ducts (40). With our modern lifestyle we are increasingly exposed to chemicals in our everyday life. Studies show that smoking, hair dye (44), nail polish (167), lipsticks, detergents, food flavorings (46), and livingplaces near superfund toxic waste (45) are risk factors for PBC.

## 6.2 Prognosis and treatment of PBC

The survival of PBC has markedly improved since the 1980s and the mostly used Mayo prognostic curve (99), made before UDCA treatment, no longer applies to the PBC population of today. Its value is limited in showing how the patients would do without any treatment and serves as a control prognostic model. It seems probable that UDCA has a positive effect on survival, even though it is not shown in meta-analyses (118,119). The reduction of need for liver transplantations with UDCA treatment was found in a Chinese meta-analysis of 7 randomized controlled trials (RTC) (118), but not in the meta-analysis of 15 RTCs (119). Recent studies from Holland (121) and Spain (122) show that patients with normal bilirubin and albumin levels on UDCA treatment have the same survival expectation as the normal population. It has also been shown that histological stage is a good prognostic factor for survival (120). The significant positive effect of UDCA has been shown in liver histology in the early stages of PBC, but not in the later stages (120,117). This finding was not confirmed in the latest meta-analysis (119). The UDCA treatment, however, does not stop the disease progression in all patients. At least 50% of patients even in the early stages (120), if not dead earlier for other reasons, will end up with liver cirrhosis and eventually suffer from decompensation or complications of cirrhosis. Today, the only approved medical therapy for PBC is UDCA,

although its position is challenged with the recent meta-analysis, and novel therapies should come on top of it.

Immunosuppressive and immunomodulatory medications have been studied in PBC, but none of them have been superior to UDCA. This was found also in a Finnish study comparing UDCA with colchicine or placebo (168). Individual patients may benefit from additional colchicine (169), but the meta-analyze did not confirm its effect (135).

Corticosteroids have been shown to improve liver histology (123–125). Prednisolone has systemic side-effects and especially bone mass density may decrease (123). Budesonide with its high first pass metabolism in the liver (127) gives the opportunity to treat the liver inflammation with less systemic effects. The budesonide 9 mg/day in combination with UDCA has been shown to improve liver histology by 30% in two years of treatment compared to UDCA alone, where the histology deteriorated by 3.5% (126). The effect on inflammation was clear, a 25% improvement in the combination group compared to a 3.2% improvement in UDCA treatment. In our study (II), we combined UDCA to a lower dose of budesonide, 6 mg/day, for three years and found a 22% improvement of stage in the combination group and 20% deterioration of stage in UDCA group. The number of patients whose stage improved or remained stable was 30/37 in the combination group and 17/32 in the UDCA group. If only improvement is considered the numbers were 16/37 and 7/32. Eight patients in the combination therapy group normalized their liver histology in three years while none of the UDCA group had such a marked improvement. The number of patients evaluated was 69, not 80 as planned, but the changes in the histology were stronger than expected so the power in our study remained strong. The improvement of the histological stage really is there, whether you evaluate the effect in an individual patient or the overall improvement in a group of patients.

Who of the patients would benefit from the combination therapy? The age at onset of PBC varies, certainly the overall impact of PBC is larger to a patient at 25 years compared with a patient over 70. The treatment activity should be individual. The treatment of PBC should always start with UDCA, but if the biochemical effect is not full the prognosis is worse in these patients compared to patients with a full biochemical response, and probably normal survival (121, 122). In our study (II), the histological inflammation was a prognostic factor for improvement of stage. If the grade was  $\geq 2$ , the effect on the stage was larger than if the inflammation was mild, grade  $\leq 1$ . In case of a partial or no response to UDCA the liver biopsy should be considered and if the inflammation is found, despite the UDCA treatment, then additional medications should be introduced. An interesting issue is the duration of the additional treatment. Would a short (1–2 years) induction therapy with budesonide be enough or do these patients need maintenance therapy with budesonide?

## 6.3 Safety of the budesonide

### 6.3.1 Pharmacokinetics

The safety aspects of corticosteroid therapy, even with budesonide, are a concern. Budesonide has a high first pass metabolism in the liver and liver diseases affect the metabolism (170). The patients with advanced liver disease, cirrhosis, are no longer candidates for budesonide therapy (170). The pharmacokinetics of budesonide have been previously studied in short-term studies (from single dose to three weeks) in PBC-patients, and there seems to be a marked difference between the early stage (I–II) PBC and the cirrhotic stage (IV). Patients with cirrhotic PBC have about 3–4 times higher plasma budesonide concentrations than patients with early stage PBC (170). In our study with 22 patients (III), no differences were observed in the AUC of budesonide in stages 0–I, II, and III. The pharmacokinetics of the three patients at stage 0 actually seemed similar to what would be expected with the present dosing in healthy subjects on the basis of a previous study about the single-dose pharmacokinetics of budesonide (171). In their study, the AUC and  $C_{\max}$  were higher in patients with early stage PBC than in healthy volunteers after a single dose of 3 mg budesonide (171). The small number of patients at stage 0 limited our possibility to find a statistical difference compared to other groups. In our study (III), the systemic effect of budesonide in patients with stages I–III was seen after two years when the serum cortisol levels started to be lower in the combination group compared to patients on UDCA alone. A clinical concern of long term use of budesonide is its metabolism by cytochrome P450. The coadministration of budesonide and CYP3A4 inhibitors, for example antifungal itraconazole, markedly increases the systemic exposure to budesonide and may lead to an increased risk to adverse effects (172).

### 6.3.2 Effects on bone

Osteoporosis and osteopenia in PBC have been an issue of controversy. It seems that PBC patients have more osteoporosis than general population, but does the stage of PBC associate with osteoporosis remains controversial. In an American study the rate of osteoporosis was 32.1 times higher in PBC patients than expected and the risk was 5.4 fold in patients at stages III and IV compared to stages I and II (138). In their study other independent risk factors were age, body mass index, and a history of fractures. The Spanish study found an increase of osteoporosis in PBC patients to be associated with age, menopausal status, BMI, and duration of PBC, but also with advanced histological stage and abnormal biochemistry (139). In the French study the PBC patients had a Z-score of -0.9 SD, meaning a lower BMD than the control population, however, they did not find an association with stage in liver histology. In their study population the only

risk factor was hormonal status. The T-score was -1 SD in premenopausal women, -1.1 SD in postmenopausal women with hormonal replacement therapy, and -2.7 SD in postmenopausal women without replacement therapy (140). The American gastroenterological association found level A evidence to support the view that the PBC patients are at risk for osteoporosis and fractures due to predominant female sex and older age, but not due to cholestatic liver disease per se (173). In a population based study from the UK, the risk for any fractures in PBC patients was a 2-fold increased compared to the general population (141). The histological stage had no effect on fracture rates. In our PBC population (Studies II and III), the baseline bone mass density was not significantly associated with the baseline stage of PBC, but the result refers to a rather mild PBC with a small number of patients at stage III and no patients at cirrhosis.

The fracture risk in the post orthotopic liver transplantation (OLT) period is high and the single most important risk factor is the pre OLT osteopenia (173). Bearing this in mind the treatment with budesonide should be extremely safe in regards to BMD. In our study (III), despite calcium and vitamin-D supplementation, bone mass density decreased in the combination therapy (FN 3.6% and LS 2.8%) somewhat more than in UDCA monotherapy (FN 1.9% and LS 0.7%), although the difference between the groups was not statistically significant. The greatest BMD reduction usually occurs during the first months of the glucocorticoid therapy (174), and our long-term therapy might alleviate the early differences between the treatment groups.

A considerable scattering exists in the effects of both treatments on the BMD values both in the FN and LS. The finding that three of the 32 patients of the budesonide-UDCA group had a 10% or greater decrease in the femoral neck BMD within 3 years is of concern. Only one of the patients using UDCA alone had an over 10% decrease in BMD. The situation in LS was better. Only one patient in the combination group had a decrease over 10% in BMD, and clinical fractures in post-OLT situation are more common in LS. Unfortunately, neither the BMD at the entry nor the stage of PBC was able to predict those patients who developed the greatest BMD changes. The number of patients with a bone density analysis in our study III was only 57, which may be inadequate to find associations between the BMD and the above mentioned predicting factors. The study with 9 mg/day of budesonide for two years (126), showed reductions of BMD in LS of 2.3% in the combination group and 1.2% in the UDCA group (they did not provide data on FN) and compared with our budesonide group their reductions were slightly smaller. Angulo et al. treated 22 patients with a combination of UDCA and budesonide, 9 mg, for one year in an open not randomized study and compared the bone density before and after the treatment and with matched controls (131). They found a marked decrease of BMD in the treatment group compared to matched controls,

and the loss of bone density was greater in cirrhotic patients compared to non-cirrhotics, and postmenopausal compared to premenopausal women (131). This study shows how important the patient selection to the individual treatments is. We know now that the cirrhotic patients are no longer candidates for budesonide therapy (170). Postmenopausal women are at a greater risk for osteoporosis even without liver disease or the use of budesonide. The transdermal estrogen therapy is shown to be effective in increasing BMD (142,143), and safe in regards to the liver (142), in postmenopausal PBC patients. The estrogen replacement therapy is recommended in early menopause and female hypogonadism, but not in older postmenopausal women (137). The hypogonadism of men should be treated with testosterone (137).

The possibility of making budesonide treatment even safer is to limit its use to men and premenopausal women, although that would raise the question of not having alternative therapies for others. Careful screening and follow up for osteoporosis is essential in PBC patients, especially for those whose liver disease is not stable and the need for additional therapies and even OLT exists. The treatment options for osteoporosis with life style changes (smoking, exercise, and calcium and vitamin-D supplementation) include raloxifene, calcitonin, and PTH (137). In recent years the studies with alendronate have shown improvement in BMD (145) and it seems superior to etidronate in patients with PBC (144). Short term safety is good, but long term safety data on PBC patients is lacking.

## 6.4 Liver transplantation and complications of cirrhosis

PBC has been the foremost indication of liver transplantation in Finland. By the end of 2006, 106 patients were grafted for PBC. Differences in causes of OLT between the Nordic countries exist. The proportion of PBC/alcohol cirrhosis/viral cirrhosis (HBV and HCV) were 11.9/13.8/3.2% in Finland, 8.3/24.3/5.3% in Denmark, 5.5/18/13.3% in Norway, and 6.6/10.2/21.1% in Sweden during the period from 1997 to 2005 ([www.scandiatransplant.org](http://www.scandiatransplant.org)). If only percentages of liver transplantations for PBC are evaluated, the Finnish transplantation rates for PBC may seem high compared to the other Nordic countries. The high percentage of PBC transplantations in Finland, however, is only relative because of low numbers of alcohol and viral cirrhotic patients in our liver transplantation population. The finding is comparable with data from the European Liver Transplant Registry ([www.eltr.org](http://www.eltr.org)), from 1988 to the end of 2005, 11% of transplantations was performed for PBC, 33% for alcohol cirrhosis, and 41% for viral cirrhosis. The Finnish transplantation numbers at the same time were 18%, 10%, and 2%.

The hepatocellular carcinoma may affect the survival of PBC patients. The risk for HCC is reported to be as high as 12.3% in stages III and IV PBC

(97). We have no data on prevalence of HCC in PBC in Finland, but it was also a marked cause of death in our epidemiological study (I) population: 309 patients died, 152 (49%) for liver related reasons, and 17 of them, 6%, from all deaths for HCC. The surveillance for HCC in autoimmune based cirrhotic patients should be considered.

Portal hypertension with esophageal varices is common in the late stages of PBC, however, some patients may already develop varices at early stages. The regular gastroscopy screening for varices gives opportunity to treat them early with either medication (propranolol) or with the rubberband ligation. The ascites formation usually occurs late in the cirrhosis of PBC and the patients require consideration for liver transplantation.

In addition to liver transplantation, the possibilities to improve the survival in PBC are the early diagnosis and active medical treatment at early stages when changes in the liver are still recoverable. Stage IV is already beyond medical therapy, stage III is beyond UDCA therapy and the risks for the budesonide therapy are increased. The optimal timing for medical therapy is at stages I and II. The majority of the PBC patients have a positive or at least stabilizing effect of the UDCA therapy on the histology. Additional medications, however, are required for UDCA non-responders. Our current knowledge of the positive effect of budesonide in PBC is based on the precirrhotic PBC patients on UDCA therapy, not especially for UDCA non-responders. The further studies should be focused on the UDCA non-responders in order to find the real additional benefit of budesonide.

## 6.5 Surrogate markers of PBC

The possibility to treat patients with medical therapy results to the desire to find out the progression of PBC already at the early stages, not only to find out when patients reach cirrhosis. Liver histology has been the gold standard for evaluating the stage, inflammation, and fibrosis in PBC. Liver biopsy, however, is costly, uncomfortable for the patient, has safety risks, and is prone to sampling errors. These matters have raised a need for surrogate markers determining progression of PBC.

So far surrogate marker studies in liver diseases have mainly concentrated on common diseases, like viral- and non-alcoholic steatohepatitis. In those numerous studies it seems clear that the cirrhotic phase and non-cirrhotic phase of the diseases are rather easy to distinguish with biochemistry, but there is a marked overlapping in surrogate markers within different non-cirrhotic phases (175–177).

The combination of age, HA, PIIINP, and TIMP1 (tissue inhibitor of matrix metalloproteinase 1) excluded fibrosis with an accuracy of 92% with ROC 0.804 (178), this study also included patients with cholestatic



liver diseases. Grading inflammation in PBC with surrogate markers have not been as successful, however, AST may have some value (166).

In our study (IV), with non-cirrhotic patients the main finding was that most of the biochemical parameters, routinely used in clinical work, do not actually correlate with the stage, fibrosis, or the inflammation. Only the AST activity turned out to be associated with the stage and the degree of fibrosis. Both HA and PIIINP levels were better in separating the different stages and fibrosis scores. The sumcombination of these parameters (AST, HA, PIIINP, and bile acids), the PBC-score, increased the differentiating capability between the non-cirrhotic stages and the scores of fibrosis up to a highly significant level ( $p < 0.0001$ ).

The PIIINP was constantly associated with the level of LPN, ALT, and GT were associated at baseline, but we could not find correlation of the AST-level and the degree of LPN previously suggested by Corpechot (166), however, the AUROC for AST to detect significant LPN was 0.746, which has clinical value. The ALT/platelet ratio and the APRI as well as the PBC-score were associated at baseline.

Bilirubin is a strong prognostic factor when evaluating the need for liver transplantation in PBC, but in our precirrhotic material it does not have a role as a surrogate marker. Bilirubin levels were equal and normal in different stages. Compared to bilirubin, the increase of bile acids is a more sensitive marker of cholestasis and it showed correlation between the different stages and scores of fibrosis in baseline measurements in our study (IV).

Plantsterols and cholestanol did not reflect the stage of PBC or the amount of fibrosis in this study. Previously they have been reported to correlate with bilirubin levels in PBC (179), but in precirrhotic stages they do not seem to give additional information.

Comparison of AUROCs between biochemical variables and scores for discriminating the stages, fibrosis, and LPN, the PBC-score and the discriminant function of the PBC-score were superior in the stage, fibrosis, and LPN compared with previously reported scores. Using a cut-off value of 66, the sensitivity was 81.4% and specificity 65.2% for classifying the stage of PBC.

The specific value of the surrogate markers will grow as they and their combinations are examined thoroughly. Already with the present information we may start to use markers in monitoring therapies, aiming to reduce the number of repeated liver biopsies. The markers could also serve as confirming factors when the possibility of sample error confounds histological results.



## 6.6 Conclusion

The prevalence and incidence of PBC are increasing in Finland along with the other western countries. The increased incidence is partly due to a better awareness of PBC and an expanded use of routine biochemistry measurements in occupational health care, but probably also the increasing exposure to the chemicals in our everyday life has an impact in introducing antigens to susceptible individuals. Together the increasing incidence and the improved survival explain the increase in prevalence.

In planning the treatment, the overall suspected effect of PBC on an individual is important. The age at onset of the disease, the disease severity, the grade of inflammation, and the response to UDCA therapy are the key issues. The diagnosis should be made at an early stage of the disease to be able to treat the inflammation and stop the progression of fibrosis. The initial treatment is UDCA, but for the UDCA non-responders the additional therapy with budesonide should be introduced. The combination of budesonide and UDCA is superior in stabilizing, or even reversing, the stage of PBC compared to UDCA alone. The safety evaluation of budesonide treatment is essential. The adrenal suppression, hyperglycemia, and reduction of BMD may occur. The medical therapy is limited to the precirrhotic stages of PBC and the liver transplantation is the only treatment option in cirrhosis, however, the need for transplantations is reducing in PBC. The transplantation results are excellent, a 95% survival rate after one year and 77% after 10 years.

Hyaluronic acid, PIIINP, AST, and bile acids may serve as surrogate markers to monitor the treatment response in the early stages of PBC. Combining these biomarkers in to a simple index may potentiate their diagnostic value and give possibilities to reduce repeated liver biopsies in patient follow up.

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